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Ewa MĘDRELA-KUDER[2] and Dariusz ROPEK[3]

MYCOLOGICAL QUALITY OF WATER WITHIN THE MUNICIPAL LANDFILL AND IN ITS SURROUNDINGS

JAKOŚĆ MIKOLOGICZNA WÓD W OBRĘBIE SKŁADOWISKA ODPADÓW KOMUNALNYCH I NA OBSZARZE PRZYLEGŁYM

Abstract: The aim of this study was to evaluate the effect of a municipal landfill on the number of microscopic fungi in surface water, groundwater and leachate within and in the surroundings of the landfill. Surface water samples were collected from the stream Malinowka and from the stream channel, groundwater samples – from 9 piezometers located around the landfill and a leachate sample – from the leachate reservoir. Water sampling was performed once per month on an annual basis (2011). The number of fungal colony forming units (CFU/cm^3) on Malt Extract Agar (MEA, Oxoid, Basingstoke, Great Britain) was determined by plating dilutions method. Based on the conducted study, the presence of microscopic fungi – *Micromycetes* was found in surface water, groundwater and leachate within and in the vicinity of the examined landfill. Large quantitative, but small qualitative differences were detected in various research sites. In terms of seasons, surface and groundwater samples were characterized by the highest abundance of fungi in summer and autumn, while the lowest abundance was recorded in winter. In the leachate samples the highest fungal abundance was recorded in the autumn-winter season, while the lowest – in spring and summer. This demonstrates the need for regular monitoring of water both within and in the areas surrounding the landfill.

Keywords: waste, municipal landfill, surface and groundwater, leachate

Introduction

Intensive development of civilization entails environmental pollution, including water. The concept of water pollution has many definitions due to the complex nature of

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[1] Department of Microbiology, University of Agriculture, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 41 81, email: rrfracze@cyf-kr.edu.pl

[2] Section of Hygiene and Health Education, University School of Physical Education in Krakow, al. Jana Pawła II 78, 31–531 Kraków, Poland, email: ewa.medrela@awf.krakow.pl

[3] Department of Agricultural Environment Protection, University of Agriculture, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 02, email: rrropek@cyf-kr.edu.pl

this phenomenon. From the perspective of water management, water pollution is a condition when the chemical composition is changed so that its direct use for domestic or industrial purposes becomes restricted or impossible. The biological context of this definition implies that the water contamination is any toxic agent towards natural water features and water organisms, creating an imbalance in the biocenosis, resulting in the removal of one or more species that are sensitive to a particular type of pollution [1].

Waste generation is inseparable part of human domestic and economic activity. In practice, the majority of waste is landfilled and only a small percentage is processed. No waste storage technology can guarantee the complete protection of the environment from the risk associated with the waste disposal, hence the need for continuous monitoring of environmental impact of landfills. Groundwater around municipal landfills is mostly threatened by leachate. Often, the pollution load in leachate is greater than in municipal sewage [2–5].

Heavy metals are the most difficult leachate contaminants to deal with. Due to their toxic character they disturb the natural biological balance of the aquatic environment and inhibit the self-purification processes of water [6, 7]. It should, however, be noted that also microbiological contamination of leachate is a constant threat to the quality of both surface water and groundwater. The number, type and distribution of waterborne microorganisms depends on numerous physical and chemical factors, including environmental conditions, season, the quality or depth of water, *etc*. These factors also affect the physiology and morphology of bacteria, fungi or cyanobacteria. Too high concentrations of some nutrients, as well as extreme temperature and pH, negatively affect the proliferation of almost all microorganisms, which have a narrow range of tolerance [3, 8].

The aim of this study was to assess the effect of the municipal landfill Barycz in Krakow on the number of microscopic fungi in surface and groundwater as well as in leachate within and in the landfill's surroundings. Identification of fungi was performed based on the analysis of macro- and microscopic features of colonies using the available fungal taxonomic manuals.

Material and methods

To perform mycological studies, the water samples were collected within and in the vicinity of the municipal waste landfill Barycz in Krakow. It is owned by the Krakow municipality and has been entrusted for operation to the Municipal Sanitation Company Ltd. It is about 3.5 km from Wieliczka in sinkholes formed at the depleted water-bearing sands of Bogucice. It is situated in the valley of a small stream Malinowka, which is a tributary of Serafa, which in turn supplies the waters of the Vistula. The area of the municipal landfill Barycz extends from south-west to north-east, its elevation is 250 m a.s.l. in the north-east and 270 m a.s.l. in the south-west. A part of this facility is located on the hill slopes and a part – in the valley. The total area of the landfill is 37 ha. It is divided into three parts; the first one was operated from the end of 1974 to 1992, the second – to February 28th 2005 and currently the third sector – an eleven hectare basin – has been operated.

Surface water samples were collected from the stream Malinowka and from the stream channel located south of the landfill (10–14), groundwater samples were collected from 9 piezometers situated around the 3rd stage of the landfill's operation sector, where waste is being deposited (1–9), while the leachate sample was collected from the leachate reservoir (15). The collection of representative water samples was performed once per month on an annual basis (2011) with a sample scoop (PB1 model, Biosphera, Poland). The location of the research sites depended on the direction of water runoff and reflected the spatial arrangement. The research sites were indicated and described in Fig. 1.

Fig. 1. Schematic layout of the research sites for water sampling at the municipal landfill Barycz in Krakow: 1–9 – groundwater research sites (piezometers), 10–14 – surface water research sites (Malinowka stream), 15 – leachate research site (working leachate reservoir)
Source: Google Earth.

After collection, the samples were transported to the laboratory of the Department of Microbiology, University of Agriculture in Krakow, where microbiological analyzes were conducted concerning the determination of fungal number per one cm^3 of water. The number of *Colony Forming Units* (CFU/cm^3) of fungi on *Malt Extract Agar* (MEA, Oxoid, Basingstoke, Great Britain) was determined by plating dilutions method. Plates with MEA were incubated for 4 days at 30 °C and then for 4 days at 22 °C. Qualitative research was conducted using the membrane filtration method. The systematic identification of fungi was performed by comparing their morphological characteristics with several taxonomic manuals [9–13].

Results and discussion

Waste deposited in landfill sites may be a source of various pollutants not only for soil but also for groundwater and surface water. Aquatic environments surrounding municipal waste storage facilities are primarily exposed to leachate-transported pollution. Landfills with improper ground sealing become the major threat, as the contaminants can infiltrate even to considerable depth and distance [14, 15]. So far, water samples in the vicinity of municipal landfills were examined only chemically, omitting the microbiological aspect [16, 17].

Recognizing the importance of this issue, the main scientific objective of the comprehensive microbiological analyzes in this study, was to assess the mycological quality of surface water, groundwater and leachate within the municipal landfill and in its surroundings. The complete research results are shown in Fig. 2 and 3. Based on these results it was concluded that the number of fungi in the examined surface water (sites 10–14) ranged from 0 to 72 CFU/cm^3. The highest mean number of fungi throughout the study period was found at the site no. 11 (23.0 CFU/cm^3) and the lowest – at the site no. 14 (14.0 CFU/cm^3). In the remaining research sites the recorded mean numbers ranged from 15 to 20 CFU/cm^3. In the case of the examined groundwater samples collected from 9 research sites (piezometers) the number of fungi ranged from 0–83 CFU/cm^3. The maximum mean number of fungi was found at the site no. 1 (27 CFU/cm^3) and the lowest – at the site no. 8 (12 CFU/cm^3). In the remaining research sites the recorded mean numbers of fungi ranged from 15 to 25 CFU/cm^3. The authors of numerous publications emphasize that the spread of contamination in groundwater depends primarily on: the water flow velocity, hydraulic gradient of water, concentration of pollutants, filtration coefficient of aquifers and soil sorption capacity. However, the possibility of spreading of microorganisms in groundwater is limited due to the relatively short period of their survival [18–20].

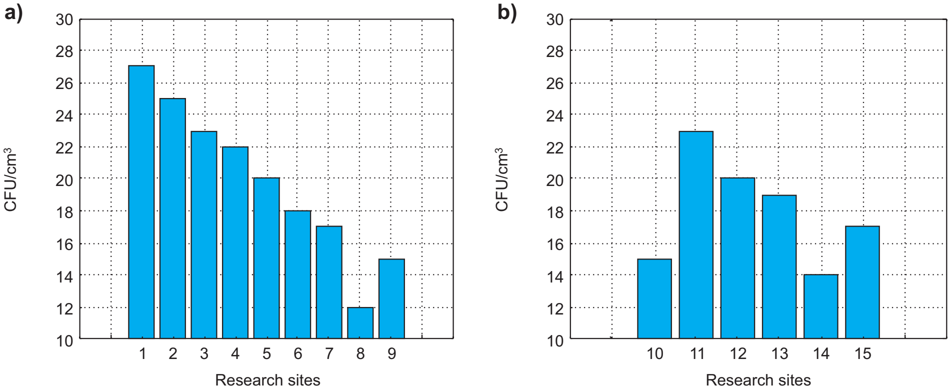

Fig. 2. Mean number of fungi (CFU/cm^3) at designated research sites within and in the vicinity of the waste landfill: a) groundwater, b) surface water and leachate

On the other hand, in the leachate sample (site 15) the number of fungi varied from 0 to 41 CFU/cm^3 and their mean number throughout the study period was 17 CFU/cm^3. Reducing the number of fungal colonies is equal to the reduction of the degrading effect of municipal landfills on the environment. It should, however, be noted that the landfilling of municipal waste inevitably leads to the formation of leachate. The amount of generated leachate depends, among others, on the type of waste, the method of storage, the period of landfill operation and the amount of precipitation. In turn, the composition of leachate depends on the stage of decomposition and on a substance contained in waste [7].

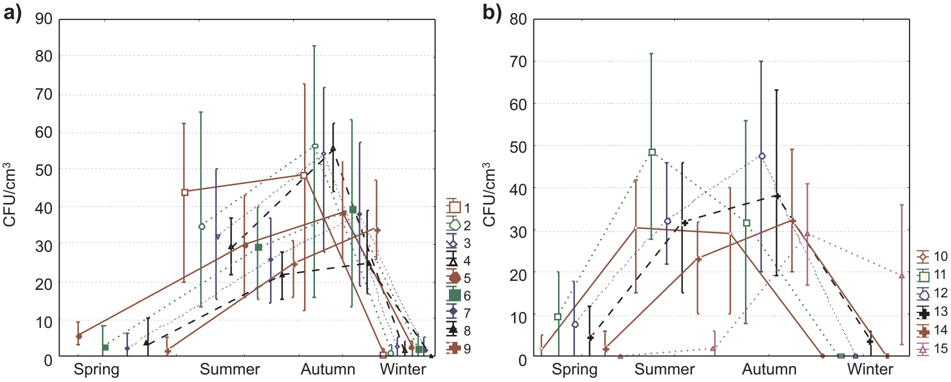

Fig. 3. Seasonal variation in the number of fungi (CFU/cm^3) (mean values; whiskers – min-max.) at the designated research sites within and in the vicinity of the waste landfill: a) groundwater, b) surface water and leachate

Figure 3 presents the number of fungi in different research periods (seasons of the year). The data obtained show that the highest mean abundance of the studied microorganisms in surface water samples was found in autumn (36 CFU/cm^3) and slightly less in summer (32 CFU/cm^3), while the lowest – in winter (1.0 CFU/cm^3). In the case of groundwater, the highest mean number of microscopic fungi was recorded in autumn (46 CFU/cm^3) and the lowest in winter – 1 CFU/cm^3. On the other hand, the highest mean abundance of fungi in leachate sample was recorded in autumn and winter (29 and 19 CFU/cm^3, respectively), while in the spring and summer seasons, the occurrence of fungi was rare. The observed relationships may result from the temperature difference and the content of organic compounds, which are one of the most important environmental factors affecting the occurrence of waterborne fungi. It should be emphasized that bacteria or fungi may grow only in a specific range of temperatures. At temperatures below zero (0 ^{o}C) water freezes and causes cell, cytoplasm or organelle damage. On the other hand, when the maximum temperature is exceeded, it causes irreversible coagulation of both structural and enzymatic proteins, and as a consequence – thermal death of microorganisms [1, 21].

The occurrence of different genera and species of waterborne fungi was also found in the examined water samples, among which *Actinospora megalospora*, *Campylospora choretocladia*, *Centrospora filiformis* and *Leptomitus lacteus* were the predominant ones. Yeast and yeast-like fungi of the genera: *Candida*, *Cryptococcus*, *Cephalosporium*, *Pichia*, *Hansenula*, *Torulopsis*, *Rhodotorula* and *Saccharomyces* were largely represented. According to Dynowska [22] the above-mentioned yeast-like fungi can tolerate large amounts of pollution and some of their species occur only in highly degraded ecosystems. Many of them are the potential etiological factors of fungal diseases, mainly in immunosuppressed patients. If these fungi have sufficient amount of nutrients in the environment, they begin to grow vigorously and constitute a very significant source of mycological infections. According to Olanczuk-Neyman [20] the increase in water pollution is accompanied by the decrease in the microbial diversity in this environment, while at the same time the number of pathogenic microorganisms increases. Nevertheless, they cannot grow or proliferate there and die eventually. However, some of them can survive for some time, whose length depends on the type of water and the prevailing conditions.

Table 1

Species composition of microscopic fungi in the water samples at the designated research sites within and in the vicinity of the waste landfill

No.	Genus/species	Surface water	Groundwater	Leachate
1	*Actinospora megalospora*	+++	+	++
2	*Anguillospora longissima*	++	++	+
3	*Campylospora chaetocladia*	+	+	++
4	*Controspora filiformis*	++	+	+
5	*Leptomitus lacteus*	+++	+	+
6	*Candida* sp.	+++	++	+++
7	*Cryptococus* sp.	+	++	++
8	*Cephalosporium* sp.	++	++	+
9	*Pichia* sp.	+	+	+
10	*Hansenula* sp.	+	+	++
11	*Torulopsis* sp.	+	++	+++
12	*Rhodotorula* sp.	++	+++	+++
13	*Saccharomyces* sp.	++	+	++
14	*Alternaria* sp.	+	+	+
15	*Aspergillus* sp.	+	++	++
16	*Botrytis cirenea*	++	++	+++
17	*Chaetomium* sp.	++	+	++
18	*Fusarium* sp.	+	++	+
19	*Penicillium* sp.	+	++	++
20	*Trichoderma* sp.	++	+	+++

Legend: +++ very frequent occurrence; ++ frequent occurrence; + rare occurrence.

Based on the conducted research, it was found that the examined water samples also contain a rich microflora of land saprophytes and ubiquitous fungi such as *Alternaria*, *Aspergillus*, *Botrytis*, *Penicillium*, *Chaetomium*, *Fusarium*, *Trichoderma*. Several of them represent soilborne allochthonous microflora, although some genera (Botrytis) may be waterborne. These fungi play an important role in the self-purification process of water. Some of them take part in the decomposition of organic acids, pectin, cellulose, hemicellulose or lignin. Some of them conduct protein ammonification or participate in the hydrolysis of fats, etc.. By mineralizing organic matter contained in water, they also contribute to the supply of nutrients to detritivorous organisms [22]. Certain species may produce antibiotic substances, probably modifying, to some extent, the quantitative and qualitative composition of waterborne microflora.

Conclusions

The following conclusions may be drawn based on the conducted analyzes of water samples:

1. The presence of microscopic fungi – *Micromycetes* was recorded in the examined surface water, groundwater and leachate samples collected within and in the vicinity of the municipal landfill Barycz in Krakow.
2. The distance of the research sites from the active landfill sector affected the number of fungi occurring both in groundwater and in surface water.
3. Large quantitative, but small qualitative differences were detected in various research sites. Depending on the season, surface and groundwater samples were characterized by the highest abundance of fungi in summer and autumn, while the lowest abundance was recorded in winter. In the leachate samples the highest fungal abundance was recorded in the autumn-winter season, while the lowest – in spring and summer.
4. Quantitative and qualitative differentiation of microscopic fungi in the examined water samples indicates the necessity for regular water monitoring both within and in the areas surrounding the landfill.

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JAKOŚĆ MIKOLOGICZNA WÓD W OBRĘBIE SKŁADOWISKA ODPADÓW KOMUNALNYCH I NA OBSZARZE PRZYLEGŁYM

Katedra Mikrobiologii
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem przeprowadzonych badań była ocena wpływu składowiska odpadów komunalnych na kształtowanie się liczebności grzybów mikroskopowych występujących w wodach powierzchniowych, podziemnych i odciekowych w jego obrębie i obszarze z nim sąsiadującym.

Próbki wód powierzchniowych pobrano z potoku Malinówka i rowu cieku wodnego, wód podziemnych z 9 piezometrów położonych wokół składowiska, a wód odciekowych ze zbiornika na odcieki. Pobranie próbek wody dokonano raz w miesiącu w cyklu rocznym (2011 r.). Liczbę jednostek tworzących kolonie (jtk/cm^3) grzybów na agarze słodowym (Malt Extract Agar, MEA, Oxoid, Basingstoke, Wielka Brytania) oznaczano metodą posiewu rozcieńczeń. Na podstawie przeprowadzonych badań stwierdzono, że na składowisku odpadów komunalnych i na terenach przyległych stwierdzono w wodach powierzchniowych, podziemnych i odciekowych występowanie grzybów mikroskopowych – *Micromycetes*. W poszczególnych stanowiskach badawczych stwierdzono duże zróżnicowanie grzybów pod względem ilościowym, natomiast niewielkie zróżnicowanie gatunkowe. W wodach powierzchniowych i podziemnych najwyższe ich liczebności odnotowano w zależności od pory roku w okresie jesiennym i letnim, a najniższe w zimie. W wodach odciekowych najwyższe liczebności grzybów stwierdzono w okresie jesienno-zimowym, natomiast najniższe w okresie wiosny i lata. Świadczy to o konieczności regularnego monitoringu wód znajdujących się na terenie składowiska, jak i na obszarze z nim sąsiadującym.

Słowa kluczowe: odpady, składowisko komunalne, wody powierzchniowe, wody podziemne, odciek

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Dariusz ROPEK[1] and Janina GOSPODAREK[1]

EFFECT OF SOIL POLLUTION WITH OIL DERIVATIVES ON THE OCCURRENCE OF ENTOMOPATHOGENIC NEMATODES

WPŁYW ZANIECZYSZCZENIE GLEBY SUBSTANCJAMI ROPOPOCHODNYMI NA WYSTĘPOWANIE NICIENI OWADOBÓJCZYCH

Abstract: The aim of the research was to evaluate the effect of oil derivatives on the occurrence of entomopatogenic nematodes during the bioremediation process. The experiment was carried out in field conditions in two series (with bioremediation and without bioremediation) at the Experimental Station in Mydlniki near Krakow. Soil was polluted with following oil derivatives: unleaded petrol, diesel oil and used engine oil (dose: 6 000 mg of fuel · kg^{-1} d.m. of soil). Unpolluted soil was used as control. The extraction of nematodes naturally occurring in the soil was done with trap method. Larvae of *Tenebrio molitor* were used to isolate nematodes. Soil samples were taken once in each season during two year experiment. Contamination of soil with oil derivatives caused significant reduction of entomopathogenic nematodes occurrence in soil environment. The most long-lasting effect on entomopathogenic nematodes population naturally occurring in soil had used engine oil and diesel oil. Application of the biopreparation into contaminated soil had a favorable effect on enthomopathogenic nematodes occurrence. On objects where biopreparation was applied enthomopathogenic nematodes were isolated significantly faster after initial contamination than on objects where only natural remediation process were carried out. Occurrence of entomopathogenic nematodes seems to be suitable indicator of bioremediation process effectiveness in soil environment contaminated with oil derivatives.

Keywords: entomopathogenic nematodes, petrol, engine oil, diesel fuel, bioremediation

Introduction

Oil derivatives as petrol or diesel oil are widely used and are often unintentionally spilled into the environment thus being an important threat to the environment [1]. Oil pollution may affect almost all living organisms present in soil [2, 3]. There are evidence that oil derivatives have an adverse effect on soil invertebrates community [4].

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[1] Department of Agricultural Environment Protection, University of Agriculture, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 02, email: rrropek@cyf-kr.edu.pl

In contaminated soil diesel oil and petrol has among others a negative impact on the nitrification process [5].

It is difficult to predict the impact of oil derivatives pollutions on soil environment. Oil derivatives may evaporate, become diluted or transformed in physical, chemical or biological processes. Durability of soil contamination with oil derivatives depends on these processes and type of these products. Contaminated soil often requires special treatment as bioremediation techniques to fasten degradation of non-volatile fractions of oil derivatives [6]. There are not many researches concerning the effect of oil derivatives contamination on soil mesofauna and especially on nematodes [2, 7–9]. However soil mesofauna is often used as an indicator of environment pollution with heavy metals [10]. Entomopathogenic nematodes commonly occurs in soil environment [11, 12] as a free-living, third stage *infective juveniles* (IJs). Nematodes are also regarded as possible indicators of the environment contamination *eg* heavy metals [13, 14]. It is important to determine the efficiency of soil restoration not only with chemical parameters but also with biological criteria [7].

The aim of the research was to evaluate the effect of oil derivatives during the process of their bioremediation on natural occurrence of entomopatogenic nematodes. It was assumed that nematodes may be sensible indicators of soil contamination with oil derivatives and effectiveness of bioremediation process.

Material and methods

The research was conducted at the Experimental Station in Mydlniki near Krakow. The experiment was set up in autumn 2009 on an agricultural land used as a hay meadow. The containers (*ca* 1 m^3) were dug into the soil at the ground level and filled with indigenous soil, and the natural soil layer arrangement was preserved. For six months the soil was left in the containers to return to its natural density and biological stability. After that period in June 2010 the soil was artificially polluted with petrol, diesel fuel and used engine oil. Oil derivatives were poured onto the surface of soil in the amount equal to 6 000 mg of fuel per 1 kg of soil dry mass. In the control unpolluted soil was placed in containers.

The experiment was set up in two variants: in the first ZB-01 preparation designed for bioremediation of soils polluted with oil derivatives was added, whereas in the second variant bioremediation process was running naturally with no preparation supplied. The biopreparation was applied several days after the soil contamination with oil derivatives. The soil bioremediation process was chemically controlled [15]. The experiment was set up in four replications. Four times a year (in each season) soil samples were collected from each container form the depth of 0–20 cm by means of Ebner's stick. The soil was placed in plastic sterile containers (120 cm^3) and than 10 larvae of trap insect *Tenebrio molitor* were put into them. Death rate of trap insects was checked every 24 hours. Dead *T. molitor* larvae were removed into sterile Petri dishes and stored at 25 ^oC for 48 hours. After two days since the test insect death they were dissected under a binocular magnifying glass to count the number of nematodes able to actively penetrate into their bodies. Rate of male and female of nematodes entering insect body was also calculated.

The results were analysed statistically using the Statistica 10,0 PL programme. ANOVA analysis was conducted and the Newman-Keuls critical intervals were computed. The value of the final step was used for differentiating means at the significance level $p < 0.05$.

Results and discussion

The occurrence of entomopathogenic nematodes in soil was performed with the use of trap insects [16]. The mortality of trap insects was observed and the cause of death was determined. High mortality of test insects on contaminated soil was observed (Fig. 1). However, no infective juveniles were found inside the insects. It suggest that the test insect death was caused by oil derivatives applied into the soil or other microorganism *eg* entomopathogenic fungi.

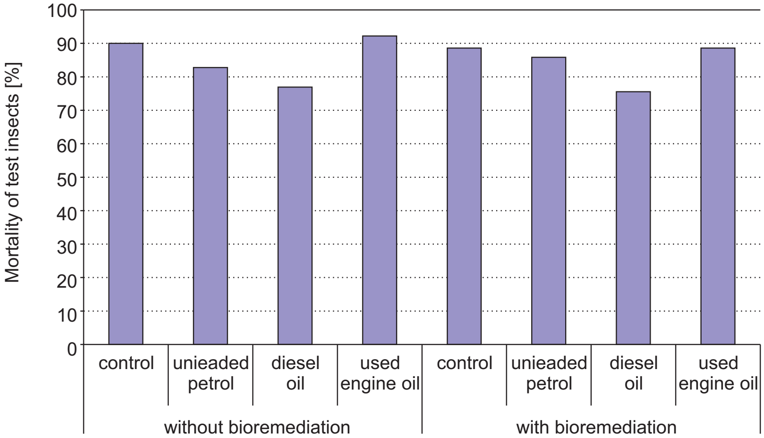

Fig. 1. Mortality of test insects – average for the investigated period

Soil samples were taken before the experiment set up to evaluate natural occurrence of entomopathogenic nematodes. It was confirmed that entomopathogenic nematodes were present in the soil where the experiment was conducted. Only one species *Steinernema feltiae* was isolated from the soil, which is one of the most often occurring entomopathogenic nematode in agricultural and urban ecosystems in Poland [11, 17]. In uncontaminated soil (control) entomopathogenic nematode was isolated in all seasons during the two year experiment (Table 1, Fig. 2). Contamination of the soil with oil derivatives significantly affected nematode occurrence. In contaminated soil nematode was not isolated for at least several months from the moment of soil pollution. The adverse effect of used engine oil application were apparent during the whole period of the experiment – no entomopathogenic nematodes were isolated from the moment of soil pollution to the end of the experiment. Soil contamination with unleaded petrol and diesel oil did not influenced entomopathogenic nematodes occurrence to the same

Table 1

The effect of soil pollution with oil derivatives on the occurrence of entomopathogenic nematodes

Date	Mortality of test insects caused by entomopathogenic nematodes [%]							
	Without bioremediation				With bioremediation			
	Control	Unleaded petrol	Diesel oil	Used engine oil	Control	Unleaded petrol	Diesel oil	Used engine oil
Spring 2010	72.5	0	0	0	70	0	0	0
Summer 2010	75	0	0	0	80	0	0	0
Autumn 2010	65	0	0	0	70	0	0	0
Winter 2011	65	0	0	0	65	0	0	0
Spring 2011	75	0	0	0	72.5	0	17.5	0
Summer 2011	80	0	0	0	77.5	0	15	0
Autumn 2011	72.5	0	15	0	67.5	0	17.5	0
Winter 2012	65	25	12.5	0	65	22.5	20	0
Spring 2012	77.5	12.5	15	0	72.5	17.5	12.5	7.5
LSD ($\alpha = 0.05$) factor date × contamination x remediation – 15.45								

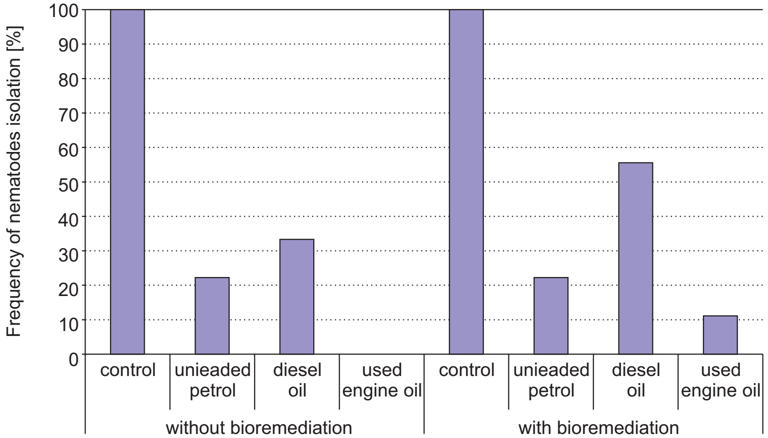

Fig. 2. Frequency of nematodes isolation from the contaminated soil – average for the investigated period

extent. In the objects contaminated with diesel oil *S. feltiae* was isolated in Autumn 2011 (15 months after contamination) and in objects with unleaded petrol in Winter 2012 (18 months after contamination). Application of the biopreparation markedly limited the adverse effect oil derivatives on entomopathogenic nematodes occurrence. This phenomena was particularly evident in objects contaminated with diesel oil.

In unpolluted objects death rate of trap insects caused by entomopathogenic nematodes was about 65–80 % (Table 1). It indicates that the population of entomopathogenic nematodes was quite numerous in the soil. The activity of nematode changed with the season and was higher in summer and lower in winter. In the objects polluted with oil derivatives mortality of trap insects caused by *S. feltiae* was significantly lower than in control and ranged from 7.7 to 25 %. Application of the biopreparation in the polluted objects caused that the trap insect were earlier infected with *S. feltiae* than in objects were bioremediation process ran naturally. However, mortality rate of trap insects in the objects with or without biopreparation supplement was very similar.

Aside from test insects mortality, also the intensity of test insects infestation by *S. feltiae* was investigated (Table 2, Fig. 3). On the uncontaminated objects without biopreparation mean intensity of trap insect infestation was 6.3–19.8 IJs and on objects with biopreparation 6.8–20.8 IJs, respectively. Application of oil derivatives caused that trap insects were not infested with entomopathogenic nematodes for at least several months after contamination. In months in which nematodes killed trap insects infestation intensity ranged 2.8–4.8 IJs (unleaded petrol) and 2.5–3.0 IJs (diesel oil). On the objects contaminated with used engine oil no entomopathogenic nematodes infested trap insects. Application of biopreparation did not influenced intensity of test insects infestation by *S. feltiae* but significantly affected time when first infective juveniles were isolated from contaminated soil.

Table 2

The effect of soil pollution with oil derivatives on the intensity of trap insect infestation with entomopathogenic nematodes

Date	Number of IJs entering test insect body							
	Without bioremediation				With bioremediation			
	Control	Unleaded petrol	Diesel oil	Used engine oil	Control	Unleaded petrol	Diesel oil	Used engine oil
Spring 2010	6.3	0.0	0.0	0.0	6.8	0.0	0.0	0.0
Summer 2010	8.8	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Autumn 2010	13.8	0.0	0.0	0.0	12.0	0.0	0.0	0.0
Winter 2011	15.5	0.0	0.0	0.0	12.8	0.0	0.0	0,0
Spring 2011	19.8	0.0	0.0	0.0	16.8	0.0	2.5	0.0
Summer 2011	18.8	0.0	0.0	0.0	20.8	0.0	4.0	0.0
Autumn 2011	13.3	0.0	2.5	0.0	12.0	0.0	2.5	0.0
Winter 2012	13.3	2.8	3.0	0.0	12.0	2.8	2.3	0.0
Spring 2012	17.3	4.8	2.8	0.0	18.3	3.5	2.8	0.3
LSD ($\alpha = 0.05$) factor date × contamination × remediation – 5.65								

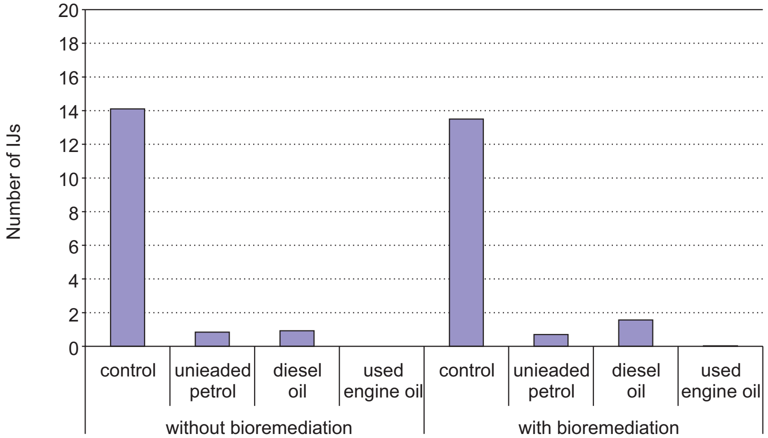

Fig. 3. Intensity of test insects infestation by entomopatogenic nematodes – average for the investigated period

Not only the intensity of test insects infestation by entomopathogenic nematodes was affected by oil derivatives but also the ratio female/male of IJs entering test insect body (Fig. 4). In contaminated soil number of male nematodes isolated from insects was rather low. In soil contaminated with oil derivatives female nematodes were more abundant and were able to infest test insects. It suggests that IJs which develop inside insects into females are less sensitive to oil derivatives than the larvae developing into males.

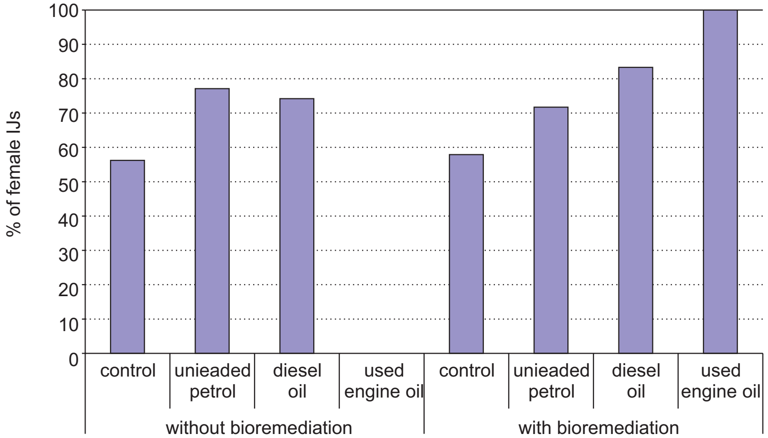

Fig. 4. Female/male ratio of *S. feltiae* isolated from dead insects in soil contaminated with oil derivatives

Entomopathogenic nematodes were not isolated from soil contamination with oil derivatives which indicates that these contaminates significantly affected soil environment. Trap insect method allows isolation of living and active IJs. The adverse effect of oil derivatives on nematodes could be associated both with death of IJs and with inability of nematodes to locate a potential host.

In soils contaminated with used engine oil entomopathogenic nematodes were isolated only on objects treated with biopreperation. However nematodes were isolated not until two years after initial contamination. On objects contaminated with unleaded petrol and diesel oil the toxic effect was less sustained. First nematodes were isolated 18 months after contamination. It indicates that among investigated oil derivatives used engine oil has most toxic effect on entomopatogenic nematodes community in the soil. Reisolation of entomopathogenic nematodes from the contaminated soil may be related with reactivation of nematode's IJs, which survived in soil or migration of new population from neighboring areas. Entomopathogenic nematodes can actively migrate in soil in search for a new host [18] or be passively carried by infested insects. As it was shown in other paper some representatives of invertebrates may penetrate soil surface shortly after initial pollution [19, 20], thus contributing to nematodes recolonization of contaminated area.

It is important to include biological indicators of soil pollution with oil derivatives. Entomopathogenic nematodes seems to be suitable indicators of bioremediation process in soil environment.

Conclusions

1. Entomopathogenic nematodes were not isolated from soil contaminated with oil derivatives for several months after application of contaminates.
2. The most negative effect on entomopathogenic nematodes had used engine oil.
3. Application of the biopreparation accelerated bioremediation process and reduced a negative effect of soil contamination with oil derivatives on the occurrence of enthomopathogenic nematodes.
4. Occurrence of entomopathogenic nematodes seems to be suitable indicator of effectiveness of bioremediation process in soil environment contaminated with oil derivatives.

Acknowledgement

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WPŁYW ZANIECZYSZCZENIA GLEBY SUBSTANCJAMI ROPOPOCHODNYMI NA WYSTĘPOWANIE NICIENI OWADOBÓJCZYCH

Katedra Ochrony Środowiska Rolniczego, Wydział Rolniczo-Ekonomiczny
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem badań było poznanie wpływu wybranych substancji ropopochodnych na występowanie nicieni owadobójczych w środowisku glebowym w trakcie procesu bioremediacji. Doświadczenie przeprowadzono w warunkach polowych w dwóch seriach (z bioremediacją i bez bioremediacji) w Stacji Doświadczalnej w Mydlnikach koło Krakowa. Gleba w każdej serii została sztucznie zanieczyszczona następującymi substancjami ropopochodnymi: benzyną, olejem napędowym i zużytym olejem silnikowym (dawka 6 000 mg paliwa · kg^{-1} s.m. gleby). Kontrolę stanowiła gleba niezanieczyszczona. Nicienie owadobójcze naturalnie występujące w glebie izolowano metodą owadów pułapkowych, przy wykorzystaniu larw mącznika młynarka. Analizę prowadzono przez okres dwóch lat, pobierając glebę do analizy w każdej porze roku. Zanieczyszczenie gleby ropopochodnymi wpłynęło negatywnie na występowanie nicieni owadobójczych w środowisku glebowym. Z gleby zanieczyszczonej substancjami ropopochodnymi nicienie owadobójcze nie były izolowane. Najbardziej długotrwały, niekorzystny wpływ na nicienie owadobójcze naturalnie występujące w glebie miało zastosowanie oleju silnikowego i oleju napędowego. Zastosowanie biopreparatu miało korzystny wpływ na występowanie nicieni owadobójczych. W obiektach, w których zastosowano biopreparat mikrobiologiczny, nicienie owadobójcze izolowano istotnie wcześniej po ich skażeniu niż w obiektach, w których proces remediacji zachodził w sposób naturalny. Analiza występowania naturalnej populacji nicieni owadobójczych w glebie może być wykorzystana do monitorowania przebiegu procesu bioremediacji gruntu zanieczyszczonego substancjami ropopochodnymi.

Słowa kluczowe: nicienie owadobójcze, benzyna, olej napędowy, olej silnikowy, bioremediacja

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Adam RADKOWSKI[1]

LEAF GREENNESS (SPAD) INDEX IN TIMOTHY-GRASS SEED PLANTATION AT DIFFERENT DOSES OF TITANIUM FOLIAR FERTILIZATION

INDEKS ZAZIELENIENIA LIŚCI (SPAD) TYMOTKI ŁĄKOWEJ W UPRAWIE NASIENNEJ W ZALEŻNOŚCI OD NAWOŻENIA DOLISTNEGO TYTANEM

Abstract: One-factor field experiment in randomized block design with four replications (plot size for harvest was 10 m^2). The experimental field covered degraded black earth soil formed from loess, class I, wheat group of very good quality, by the agricultural soil classification system. The experiment was conducted at the Plant Breeding Station of Malopolska Plant Growing Company – HBP LLC in Skrzeszowice near Krakow (200 m a.s.l.) from 2006 to 2009. The experimental factor was spraying with titanium fertilizer labelled Tytanit at three different concentrations: 0.02, 0.04 and 0.08 %. During growing periods chlorophyll content was measured by means of SPAD readings. Relative chlorophyll concentration indices were growing from shoot elongation stage to anthesis stage, when the highest value was obtained, whereas at milky ripe stage SPAD readings diminished to the level prior to shoot elongation. Foliar fertilization with Tytanit applied at 0.08 % concentration resulted in the highest increase in SPAD value (the difference averaged 6 % over plants from the control object). Again, plants form the objects treated with the most diluted preparation (0.02 %) had the least chlorophyll content over the control one (difference of about 2 %).

Keywords: timothy-grass, Skald cultivar, titanium, SPAD

Introduction

In modern agricultural production the use of growth stimulants alone, or as additives in solutions of foliar fertilizers, is a factor which enable to reduce detrimental effect of frost, ground chills or drought [1–3]. Such a task can be fulfilled by titanium. This is one of the most important microelements, which exerts positive effect on the biochemistry of plants as regards processes leading to earlier and highest crop production [4]. Titanium feeding stimulates activity in many enzymes, *eg* catalase, peroxydase, lipoxygenase or nitrate reductase. Additionally, it increases some metabolic

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[1] Department of Grassland, Agricultural University of Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 43 59, fax: +48 12 633 62 45, email: rrradkow@cyf-kr.edu.pl

processes, encourages pollination, fecundation and development of seed and fruit. Titanium leads to higher chlorophyll content in leaves, augments their growth and development. This element also enhances growth and development processes in plants, make them less vulnerable to adverse environmental conditions, improves their resistance against fungal and bacterial diseases. Titanium feeding has shown beneficial influence on the uptake of other nutrients, both from soil and from fertilizers [2, 5, 6]. Investigation into the effects of titanium on plant yielding has been conducted mainly in vegetables and field crops, and their results has confirmed the positive outcomes of titanium feeding. However, there is hardly any reports addressing the consequences of titanium fertilization for pasturable grass yield in a seed plantation. Timothy-grass, when grown for seeds, is a species that requires special attendance in terms of nitrogen fertilization in early development. The species gives a positive response to higher nitrogen concentration in soil and produces numerous vegetative shoots at the expense of generative organs [7]. Consequently, it is recommended to divide nitrogen supply into smaller doses. Due to the use of a SPAD meter one can immediately notice any undesirable changes in plant condition. Measurements proceeded by the chlorophyll content meter are non-destructive; the head of the instrument is placed over the sample of plant tissue and approximately by 2 seconds one can obtain an estimated value of chlorophyll concentration. Currently an increasing number of foliar fertilizers offered on the market contains slight amounts of titanium.

The field studies aimed at finding changes in chlorophyll content in timothy-grass of Skald cultivar grown for seed as influenced by foliar titanium feeding with commercial fertilizer labelled Tytanit at three different Tytanit concentrations: 0.02, 0.04 and 0.08 %.

Materials and methods

A field experiment was conducted at the Plant Breeding Station in Skrzeszowice, which belongs to Malopolska Plant Growing Company – HBP LLC, Krakow, from 2006 to 2009. The experiment was established according to randomized block design with four replications and located over degraded black earth formed from loess; according to the agricultural soil classification system the soil was assigned to class I, wheat group of very good quality. Its chemical properties were as follows: pH_{KCl} – 7.32, P_2O_5 – 190 mg, K_2O – 155 mg and Mg – 69 mg of available nutrient per 1 kg of soil. The soil was moderately rich in available manganese and zinc, while available forms of copper were at low concentration.

Water and temperature conditions in years from 2006 to 2009 against multi-year averages (1991–2004) are presented in Table 1. As can be seen from these data each constellation of meteorological circumstances in the individual experimental years was different.

During the growing seasons (April to September) in year 2006, 2007, 2008 and 2009 total precipitation amounted to 325.0, 465.0, 377.7, and 359.6 mm respectively, while respective mean air temperatures were 13.9. 12.5. 11.7 and 16.1 ^{o}C. More specifically we can describe that in 2006 rainfall occurrence was irregular over growing season. From the average values of Sielianinov coefficient one may say that fairly wet

Table 1

Hydrologic and thermal conditions for years 2006 to 2009 compared with multi-year averages (1991–2004) for Plant Breeding Station in Skrzeszowice

Month	Year				
	2006	2007	2008	2009	1991–2004
Precipitation [mm]					
IV	41.5	27.0	47.0	0.4	51.8
V	65.0	67.0	32.0	94.5	68.0
VI	64.0	84.0	53.5	123.1	74.9
VII	31.0	60.0	144.5	69.9	92.4
VIII	101.5	64.0	39.0	49.0	65.2
IX	22.0	163.0	61.7	22.7	56.3
X	14.0	54.0	46.4	65.0	49.6
XI	57.0	34.0	15.5	79.2	30.4
Total	396.0	553.0	439.6	503.8	488.6
Average air temperature [^{o}C]					
IV	7.8	5.4	4.6	11.4	8.6
V	12.5	11.5	9.8	14.2	14.3
VI	15.4	16.6	15.2	16.8	17.2
VII	19.0	16.6	15.5	20.4	19.1
VIII	15.9	15.5	14.8	18.8	18.6
IX	13.0	9.4	10.0	15.1	13.2
X	6.4	4.7	8.1	7.9	8.6
XI	5.1	0.5	3.9	5.2	3.3
Mean	11.9	10.0	10.2	13.7	12.9
Sielianinov hydrothermal coefficient					
IV	1.8	1.7	3.4	0.0	2.0
V	1.7	1.9	1.1	2.1	1.5
VI	1.4	1.7	1.2	2.4	1.5
VII	0.5	1.2	3.0	1.1	1.6
VIII	2.1	1.3	0.9	0.8	1.1
IX	0.6	5.8	2.1	0.5	1.4
X	0.7	3.7	1.8	2.7	1.9

conditions prevailed in April and May, optimum ones in June, then July was very dry, August wet, while September very dry again. Total precipitation in August amounted 101.5 mm and it was 36.3 mm higher in relation to multi-year average. In 2007 meteorological circumstances during growing season were characterized by lower air temperature (by 2.9 ^{o}C) and higher precipitation (by 64.4 mm) than in years 1991–2004. Relatively wet conditions occurred in April, May and June, fairly dry ones in July and August, whereas extremely wet in September and October. The highest amounts of rainfall were noted in September (163.0 mm), high precipitation was observed in June (84.0 mm), May (67.0 mm) and August (64.0 mm), while the lowest one in April (27.0 mm). In 2008 air temperature was lower (by 2.7 ^{o}C) than multi-year average, the same was true for precipitation (by 49.0 mm). From calculated values of Sielianinov hydrothermal coefficient it is possible to conclude that April and July were extremely wet months. In 2009 meteorological circumstances during growing season were characterized by higher air temperature (by 0.8 ^{o}C) and higher precipitation (by 15.2 mm) in relation to multi-year average. The values of hydrothermal coefficient indicate at wet conditions in May and June, although extremely dry in April.

The seeds were sown in rows, distance between the rows being 40 cm, and amount of seeds required was 4 kg $\cdot$ ha^{-1}, without protection of companion plants.

Soil mineral fertilization was dosed as follows. In the year of sowing pre-seed fertilizers were broadcast, *eg* 50 kgN $\cdot$ ha^{-1} as ammonium nitrate, 60 kgP_2O_5 $\cdot$ ha^{-1} as triple superphosphate, and 80 kgK_2O $\cdot$ ha^{-1} as 57 % potassium chloride. Additionally in autumn phosphorus and potassium fertilizers were top-dressed in the same quantities as applied in spring. For each year of usage phosphorus-and-potassium application in spring was the same (60 kgP_2O_5 $\cdot$ ha^{-1} as triple superphosphate and 80 kgK_2O $\cdot$ ha^{-1} as 57 % potassium chloride), whereas nitrogen application rate was 100 kgN $\cdot$ ha^{-1}.

Foliar feeding was proceeded with the use of a fertilizer named Tytanit at three different concentrations: 0.02, 0.04 and 0.08 %. Tytanium content in the fertilizer is 8.5 g per litre. The first foliar spray was applied at shoot elongation stage, and the next took place 2 weeks afterward. In case of massive weed expansion Chwastox Extra 330 SL, Aminopielik D, Starane 250 SL and Gold 450 EC at the doses of 1.5, 1.5, 0.6, and 1.5 dm^3 $\cdot$ ha^{-1} respectively were employed. Additionally, the experimental field was treated with pre-emergent spray against pests with the aid of Owadofos 50 solution (1–2 dm^3 $\cdot$ ha^{-1}). The first spray was done in early springtime, when the plants started growing, and the second took place after ear emergency, but still before anthesis.

For each year of the study the influence of foliar titanium fertilization on chlorophyll content was analyzed. Leaf greenness (SPAD) indices were measured with a Minolta SPAD 502 DL chlorophyll meter in upper leaves, at four stages of development, *eg* shoot growth, ear formation, flowering and milky ripeness. Measurements were done using thirty fully developed leaves from each plot.

Obtained data underwent statistical analysis with the use of the ANOVA procedure. The values of confidence limits were found by means of Tukey's test at the confidence level of $\alpha = 0.05$. Statistical description covered the values of SPAD index.

Results and discussion

Leaf greenness indices (SPAD values) in examined timothy-grass plants of Skald cultivar ranged between 38.75 and 45.53 (Table 2) depending on measuring time. The study demonstrated that foliar application of Tytanit fertilizer at different concentrations (0.02, 0.04 and 0.08 %) had significant impact on leaf greenness index (SPAD) values over head formation, anthesis and milky ripe kernel stages. Relative chlorophyll concentration was growing with plant development up to the anthesis stage. Similar tendency was observed for the respective developmental stages, albeit in winter triticale of Woltario cultivar, by Jaskiewicz [8] and to a certain extent in rape by Kulig et al [9].

Table 2

Leaf greenness (SPAD) index in timothy-grass of Skald cultivar over stages of development and with different levels of Tytanit fertilizer (mean for four years)

Tytanit concentration	Developmental stage			
	Shoot elongation	Head emergency	Anthesis	Milky ripe kernels
Control	38.75	39.64	41.45	39.03
0.02 %	39.38	39.79	43.27	39.64
0.04 %	39.49	40.49	44.48	40.05
0.08 %	39.93	41.25	45.53	41.10
$LSD_{0.05}$	ns	0.42	1.19	0.59

Leaf greenness index reached the highest values at the anthesis stage, while at the milky ripe kernel stage it diminished again to the level prior to head emergency.

From the descriptive statistics of our data it can be seen that over the analyzed developmental stages SPAD values showed low coefficients of variations (Table 3). Average SPAD values ranged between 39.39 and 43.68, which corresponds with good growing conditions in this experiment.

Table 3

Descriptive statistics of SPAD index values in timothy-grass of Skald cultivar over stages of development (mean for four years)

Developmental stage	Range		Mean	Standard deviation	Coefficient of variation
	min	max			
Shoot elongation	38.78	41.33	39.39	2.21	5.61
Head emergency	39.25	42.02	40.29	1.62	4.01
Anthesis	40.28	45.32	43.68	1.49	3.40
Milky ripe kernels	39.02	41.94	39.96	1.24	3.10

The plants fertilized with Tytanit at the 0.02 % concentration appeared to have the lowest SPAD values. Conversely, at the highest Tytanit concentration (0.08 %) leaf greenness index reached maximum. Positive influence of foliar feeding with certain fertilizer on crop yield in plants was described by some authors including Faber et al [10], Czuba et al [11], Szewczuk [12] as well as Kocon and Grenda [13]. These works has demonstrated that after treating plants with a mixture containing titanium ions chlorophyll content in leaves was 16 to 65 % higher compared with control plants. Beneficial effect of titanium ions in plants is attributable to at least four mechanisms: augmented activity of oxidation-reduction reactions, accelerated nutrient uptake, more vigorous pollen grains and enhanced resistance against certain fungal pathogens. It has been demonstrated in literature that foliar feeding with titanium led to an increase in plant chlorophyll content; on the other hand chlorophyll synthesis is stimulated by iron ions. One may come to conclusion that it is due to improved bioactivity of iron, which is responsible for enzyme synthesis, that plant photosynthesis will be encouraged [4, 14].

Differentiated SPAD readings among distinct stages of plant development was shown by Fox et al [15] for winter wheat, Jaskiewicz [8] for winter triticale or Kulig et al [9] for rape. In the environment with light soils Rozbicki and Samborski [16] found optimal SPAD values for winter triticale to be lower. An investigation conducted by Samborski [17] suggests that the most accurate evaluation concerning nitrogen feeding in plants can be performed at the beginning of shoot elongation stage and boot stage (initial head formation).

SPAD readings obtained in this study were high, in particular for plants receiving application of the highest titanium concentration. Soil acidity/alkalinity measure was neutral (pH_{KCl} = 7.32), which improved the efficiency of spraying. According to relevant literature, natural titanium availability and uptake by plants is determined mainly by soil pH value as well as by the amount of organic substance in soil. With growing pH value and organic matter content the availability of titanium ions decreases [13].

On the basis of calculated regression equations it was possible to confirm linear correlation between seed production and leaf greenness index (Table 4). Coefficient of determination R^2 would indicate rather strong relationship between seed production and SPAD value, especially at anthesis and milky ripe stages.

Table 4

Regressive relationship between timothy-grass yield
and SPAD index over stages of development

Developmental stage	Regression equation	R^2
Shoot elongation	$y = 0.9536x + 32.34$	0.69
Head emergency	$y = 1.2434x + 31.097$	0.63
Anthesis	$y = 1.2867x + 34.172$	0.85
Milky ripe kernels	$y = 1.4547x + 29.209$	0.87

According to Faber et al [10] positive outcomes of foliar plant feeding can be accounted for by the effect of stimulated plant metabolism, so that nutrient uptake in the root system is enhanced. Moreover, these findings are supported with the reports from many authors [5, 10, 13, 18–20], which showed that foliar feeding in wheat, rape and other plants had positive impact on photosynthesis and effective nitrogen utilization, and this finally led to higher crop of these plants. Obtained positive effect of titanium on leaf greenness index (or chlorophyll content) substantiates foliar feeding of timothy-grass seed plantation as advisable, even in such tillage conditions, where one may believe that the availability of nutrients must be satisfactory. The specific conditions here, as it was presented previously in the methodological section, included degraded black earth formed from loess; by the agricultural soil classification system it was class I, or wheat group of very good quality.

Conclusions

1. Application of Tytanit fertilizer in three different concentrations had a significant influence on leaf greenness (SPAD) index. SPAD values were increasing with Tytanit concentration.
2. Relative chlorophyll concentration index was growing with the stages of plant development. The highest levels were demonstrated at the anthesis stage, while at the milky ripe kernels stage they diminished to those prior to head emergency.
3. Higher concentration of the solution (0.08 %) led to utmost increase in SPAD readings (averaged 6 %) in relation to the control object.

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INDEKS ZAZIELENIENIA LIŚCI (SPAD) TYMOTKI ŁĄKOWEJ W UPRAWIE NASIENNEJ W ZALEŻNOŚCI OD NAWOŻENIA DOLISTNEGO TYTANEM

Instytut Produkcji Roślinnej, Zakład Łąkarstwa
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Jednoczynnikowe doświadczenie polowe założono metodą losowanych bloków, w czterech powtórzeniach (powierzchnia poletka do zbioru wynosiła 10 m^2). Na polu doświadczalnym występował czarnoziem zdegradowany wytworzony z lessu, należący do I klasy bonitacyjnej, kompleksu przydatności rolniczej pszennego bardzo dobrego. Doświadczenie prowadzono w latach 2006–2009 w Stacji Hodowli Roślin należącej do Małopolskiej Hodowli Roślin – HBP Kraków w Skrzeszowicach pod Krakowem (200 m n.p.m.). Czynnikiem doświadczenia był oprysk tytanem w formie nawozu Tytanit w trzech stężeniach: 0,02, 0,04 i 0,08 %. W okresie wegetacji dokonano pomiaru zawartości chlorofilu w postaci odczytów SPAD. Wskaźnik względnej zawartości chlorofilu wzrastał od fazy strzelania w źdźbło do fazy kwitnienia, gdzie uzyskano największą wartość, natomiast w fazie dojrzałości mlecznej wartość odczytu SPAD zmalała do poziomu sprzed kłoszenia. Zastosowane nawożenie dolistne w stężeniu 0,08 % Tytanitu spowodowało najwyższy wzrost wartości SPAD (różnica wynosiła średnio 6 % w stosunku do roślin z obiektu kontrolnego). Z kolei rośliny z obiektów, gdzie wykonano oprysk o najmniejszym stężeniu (0,02 %) miały poza obiektem kontrolnym najniższą zawartość chlorofilu (różnica na poziomie 2 %).

Słowa kluczowe: tymotka łąkowa, odmiana Skald, tytan, SPAD

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Adam T. WOJDYŁA[1]

EFFECT OF VEGETABLE AND MINERAL OILS ON THE DEVELOPMENT OF *Diplocarpon rosae* Wolf. – THE CAUSAL AGENT OF BLACK SPOT OF ROSE

WPŁYW OLEI ROŚLINNYCH I MINERALNYCH NA ROZWÓJ *Diplocarpon rosae* Wolf. SPRAWCĘ CZARNEJ PLAMISTOŚCI RÓŻY

Abstract: Experiments were carried out to determine the effect of vegetable oils: corn, olive, rapeseed, sunflower, soybean, and grape; vegetable oils recommended as adjuvants: Dedal 90 EC (90 % vegetable oil) and Olejan 80 EC (85 % rapeseed oil); and mineral oils: Atpolan 80 EC (76 % SN oil), Ikar 95 EC (95 % SAE petroleum oil), Olemix 84 EC (84 % DSA petroleum oil), Promanal 60 EC (60 % petroleum oil), used at a concentration of 1 % for spraying rose bushes 9 times at 7-day intervals in order to control the black spot disease caused by *Diplocarpon rosae*.

The results do not allow the drawing of a clear conclusion as to which of the tested vegetable or mineral oils showed the highest effectiveness in inhibiting the development of the symptoms of black spot and the fungus *D. rosae*. It was found that the percentage effectiveness of the tested oils relative to the control depended on the type of oil, the time of observation and the experiment. Depending on the experiment and the time of observation, different percentage rates of the inhibition in the development of disease symptoms were obtained. After spraying the roses 3 times, the effectiveness of the tested oils ranged from 35.8 % (sunflower oil) to 77.8 % (Atpolan 80 EC); after 6 applications, it was from 26.8 % (olive oil) to 55 % (Olejan 85 EC); and after a total of 9 treatments, it was from 29.5 % (corn oil, soybean oil and Olemix 84 EC) to 58.6 % (olive oil). There was no evidence of phytotoxicity of the tested oils to the variety of rose on which the experiments were conducted.

Keywords: *Diplocarpon rosae*, vegetable and mineral oils, control, rose

Introduction

In the cultivation of roses in open field and under cover, one of the most serious and most common diseases of *Rosa thea* × *hybrida* is the black spot disease caused by the fungus *Diplocarpon rosae* Wolf. In susceptible varieties, already in May, brown spots

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[1] Department of Ornamental Plant Protection, Research Institute of Horticulture, ul. Konstytucji 3 Maja 1/3, 96–100 Skierniewice, Poland, phone: +48 46 834 55 37, email: Adam.Wojdyla@inhort.pl

appear on the lower leaves, around which yellow tissues can be seen. Before long, the diseased leaves fall off, and, in the absence of protection, in the second half of summer the affected rose bushes can become completely devoid of foliage. In the commercially available plant material there are several varieties and species of roses that are low-susceptible or resistant to the pathogen. However, market demands in relation to the quality and quantity of cut flowers produced per unit surface area make it inevitable that the cultivated crops include a number of varieties and species susceptible or very susceptible to black spot [1]. The basic method of controlling the disease relies on the use of chemical plant protection products. Literature data on the control of black spot indicate high effectiveness of synthetic fungicides, biopreparations such as garlic juice, chitosan, grapefruit extract the product Atonik stimulating plant growth and foliar fertilizers.

In the last century, pesticides were largely adopted to counteract the action of pests and disease, and to increase plant health and yield. However, the continuous use of chemical fungicides for plant defence caused great environmental impact, the onset of resistance phenomena within some populations of fungal pathogens as well as acute and general toxicity to humans and non-target organisms [2]. Therefore, oils whose mechanism of action is different from that of the previously used means could prove highly useful for controlling plant pathogens. Natural oil-based fungicides have excellent spreading and leaf-surface adhesion characteristics, and because of their rapid biodegradation have a low toxicity to people and cause little environmental impact [2].

Adjuvants, among them vegetable and mineral oils, can be used as a means of enhancing the action of the active ingredients of herbicides [3, 4], fungicides [5–7], insecticides [8], and fertilizers [9–12]. However, any improvement in the effectiveness of fungicides may depend on their formulation [5].

The author's own experiments on ornamental plants, as well as those of other researchers on fruit plants or vegetables, have shown high effectiveness of oils in controlling the pathogens that are the causal agents of powdery mildew [13, 14], leaf spot [15, 16], rust [17, 18] and grey mould [19]. Therefore, in the protection of roses against black spot, vegetable and mineral oils may prove useful, in addition to the already mentioned fungicides and bio-preparations. Oils might be used as alternatives to conventional fungicides and integrated into programmes that already include other necessary materials, thereby reducing the frequency of use of all the fungicide groups in the programme and the risk of developing resistance to them [13]. Oils are considered to be the oldest natural pesticides, as documented by a Roman scholar in the 1st century [20] cited by Jee et al [21].

According to the literature, various oils have also been used to control insect and mite pests for hundreds of years. The following pests are good candidates for being controlled by oil sprays: pine needle scale, oystershell scale, euonymus scale, aphids, spider mites, and small pine sawfly larvae [22].

The mechanism of action of vegetable oils consists in acting directly and indirectly on pathogens. Mineral oils, on the other hand, act mainly in a direct way [7]. Direct action involves dehydration of fungal cells and often disintegration of the cell walls [7].

By contrast, indirect action is associated with the induction of resistance in the plants being protected.

Among the many elicitors of *induced systemic resistance* (ISR) in plants listed in the literature there are also oleic acid, linoleic acid, and linolenic acid – the main components of the vegetable oils used in the present experiments [23] (Table 1).

Table 1

Percent by weight of total fatty acids

Oil	Unsaturated/ saturated ratio	Saturated		Mono unsaturated	Poly unsaturated	
		Palmitic ccid C16:0	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid (ω 6) C18:2	Alpha linolenic acid (ω3) C18:3
Canola oil	15.7	4	2	62	22	10
Corn oil (maize oil)	6.7	11	2	28	58	1
Grape seed oil	7.3	8	4	15	73	—
Olive oil	4.6	13	3	71	10	1
Soybean oil	5.7	11	4	24	54	7
Sunflower oil	7.3	11	5	28	51	5

Source: http://www.scientificpsychic.com/fitness/fattyacidsl.html

Studies conducted so far have shown that ISR is most effective against fungi, less effective against bacteria and least effective against systemic viruses [24]. In an experiment by Cohen et al [25], five unsaturated fatty acids were tested for their ability to induce systemic resistance in potato plants to the late blight fungus *Phytophthora infestans* (Mont.) de Bary. Linoleic acid, linolenic acid and oleic acid applied to leaves 1–3 of potato plants at a dose of about 1 mg per plant induced (provided) 82 %, 39 % and 42 % protection in leaves 4–11, respectively. Also Clayton et al [26] cited by Northover and Schneider [15] used multiple-spray programmes to protect tobacco seedlings from blue mould (*Peronospora tabacina* D.B. Adam) and found that vegetable oils with high proportions of linoleic acid ($C_{18:2}$) or other polyunsaturated acids were fungicidal, whereas oils with high proportions of the monounsaturated oleic acid ($C_{18:1}$) were nonfungicidal. These results have not always been confirmed by other researchers. In experiments by Northover and Schneider [15] there was no difference in fungicidal activity between two groups of oils with compositions either high or low in linoleic acid toward three foliar pathogens: *Podosphaera leucotricha* (Ell. et Ev.) Salmon, *Venturia inaequalis* (Cooke) Winter) and *Albugo occidentalis* G.W. Wils. Calpouzos [27] and Whiteside [28] considered it likely that oil exerted a therapeutic action not directly on the pathogens, but rather through an alteration in the physiology of the host. On the other hand, Northover and Schneider [13] found that the regrowth and reappearance of powdery mildew lesions after treatment with plant oils, and to a lesser extent with petroleum oils, showed that these oils had a fungistatic rather than fungicidal effect, possibly indicating a temporary effect on host physiology.

The effect of oils on the practical value of the resulting crop can also be very significant. Roszyk et al [29] found that rapeseed oil and the mineral oil Atpolan used as additions to the working solution of foliar fertilizers significantly increased the yield of greenhouse tomato. Oils have also been used in fruit and vegetable storage. Brooks et al [30] reported that fruit wrapped in tissue paper containing 15 % mineral oil developed much less scald after cold storage.

In case of a wrong selection of oils in relation to the time of year and the plant species to be protected, the problem of phytotoxicity may occur. The results of phytotoxicity tests obtained by different researchers may be contradictory, depending on the oils used, their concentrations and weather conditions during their application. Rongai et al [2] demonstrated that vegetable oils showed no phytotoxicity, while the formulations based on mineral oils showed a significantly lower fresh and dry weight of tomato plants. The use of oils for spraying plants during the growing season can affect plant photosynthesis and respiration. Wedding et al [31] found that the application of petroleum oil emulsions depressed the rate of photosynthesis in orange and lemon leaves. Some inhibition of the process persisted as long as determinations were made (59 days). A corresponding but smaller decrease in respiration was found in those oil-treated leaves. Other data suggested that at least part of the decrease in the percentage of soluble solids (sugars and citric acid) in the juice of fruits associated with oil-spray applications might be due to the inhibition of photosynthesis. Those results were not confirmed by later studies on rose. In experiments by Goszczynski and Tomczyk [32] on rose shrubs, the intensity of photosynthesis and dark respiration of leaves were measured 24 hours and 3 weeks after the application of Sunspray 850 EC (Ultra Fine) oil. No significant effect of 1 % and 2 % oil solutions on the intensity of photosynthesis in mature rose leaves was found 24 hours after spraying. The resistance of the stomata of oil-treated rose leaves remained unchanged.

The aim of the experiments was to determine the effect of vegetable and mineral oils on the development of Diplocarpon rosae Wolf. – the causal agent of black spot of rose.

Materials and methods

The following compounds were used in the experiments:

– **Vegetable oils used as food:** corn seed oil, olive (fruit) oil, rapeseed oil (canola oil), soya seed oil, and sunflower (seed) oil.

– **Vegetable oils recommended for plant protection:** Dedal 90 EC (90 % vegetable oil) – produced by Danmark Lodz Poland, Olejan 80 EC (85 % rapeseed oil) – produced by Danmark Lodz Poland.

– **Mineral oils:** Atpolan 80 EC (76 % SN oil) – produced by Agromix Niepołomice Poland, Ikar 95 EC (95 % SAE petroleum oil) – produced by Danmark Lodz Poland, Olemix 84 EC (84 % DSA petroleum oil) – produced by Danmark Lodz Poland, Promanal 60 EC (60 % petroleum oil) – produced by Neudorff GmbH KG Germany, Sunspray 850 EC (85 % mineral oil) – produced by Sun Oil Company (Belgium).

– **Fungicides:** Saprol 190 EC (190 g triforine per dm^3) produced by American Cyanamid Company USA, and Score 250 EC (250 g difenoconazole per dm^3) produced by Syngenta Crop Protection AG, Switzerland.

– **Surfactant:** Tergitol™ 15 – S-9) produced by DOW Chemical Co.

The experiments were carried out on roses cv. 'Red Berlin', grown in an open field and susceptible to black spot. During the course of the experiments the roses were watered using a capillary system to prevent the applied agents from being washed off the surface of the plants during sprinkling. When the symptoms of black spot (*D. rosae*) were found on the plants, a total of 9 spray treatments were carried out at 7-day intervals with oils at a concentration of 1 %. In all the experiments, shrubs were sprayed in the morning using 1 dm^3 of working solution per 10 m^2 of surface area. Both the upper and lower surface of the leaf blade was thoroughly covered. Tergitol™, at a concentration of 0.3 %, was added to the spray mixtures of the vegetable oils used. The effectiveness of the tested oils (the severity of infection) was determined using a ranking scale before the experiment and after 3 days from the execution of the 3rd, 6th and 9th spray treatment (Table 2).

A total of three series of experiments were carried out in consecutive years at different initial severity of disease symptoms and different weather conditions. Score 250 EC (difenoconazole) or Saprol 190 EC (triforine) was used as the standard (reference) fungicide. Using a formula, the percentage effectiveness of the oils in inhibiting the development of black spot was calculated [33].

The experiment was set up in a randomised block design with 4 replications, each replication consisting of 5 plants.

Results

In 2004, after the 3rd spray treatment of the roses, the percentage effectiveness of the tested oils ranged from 59.3 % (corn oil) to 77.8 % (Atpolan 80 EC). After spraying the shrubs 6 times, the effectiveness of the tested oils decreased slightly and ranged from 40 % (Promanal 60 EC) to 55 % (Olejan 80 EC). After the 9th treatment, the effectiveness of the tested oils relative to the control roses was similar and ranged from 34.6 % (grape seed oil) to 55.8 % (corn oil, sunflower oil). Neither after the 3rd nor after the 6th treatment was there any leaf drop observed, meaning there was no drastic decrease in the decorative value of the shrubs. The tested vegetable and mineral oils showed a significantly lower rate of effectiveness compared with the fungicide Saprol 190 EC (Table 2).

In 2005, after spraying the roses 3 times, the percentage effectiveness of the tested oils was from 37 % (Ikar 95 EC, Promanal 60 EC) to 62.9 % (canola oil). After the 6th treatment, the effectiveness of the tested oils decreased slightly and ranged from 35.5 % (grape seed oil) to 51.6 % (Olejan 80 EC). After spraying the plants 9 times, the effectiveness of the tested oils relative to the control roses increased slightly and ranged from 47.1 % (Olemix 84 EC) to 58.6 % (corn oil and olive oil). There was no leaf drop observed after the 3rd and 6th treatment (except for grape seed oil) and consequently no drastic reduction in the decorative value of the plants. The tested vegetable and mineral oils showed a significantly lower effectiveness in comparison with the fungicide Saprol 190 EC (Table 3).

Table 2

Effectiveness of some oils in the control of *Diplocarpon rosae* Wolf.
on roses cv. Red Berlin. Mean degree of shoots infection.
Beginning of experiment and initial disease level: 2004.06.01 = 0.1

Treatment	Conc. in %	After sprayings		
		3	6	9
Control	—	1.35 f	3.00 g	5.20 g
Saprol 190 EC	0.15	0.15 a	0.40 a	1.00 a
Plant oils used as a food (Cooking oil)				
Corn oil (maize oil)	1.0	0.55 e	1.60 de	2.40 b
Grape seed oil	1.0	0.40 b–d	1.70 ef	3.40 f
Olive oil	1.0	0.35 bc	1.60 de	2.35 b
Rape oil (canola oil)	1.0	0.40 b–d	1.60 de	2.30 b
Soybean oil	1.0	0.35 bc	1.65 e	2.45 b
Sunflower oil	1.0	0.45 c–e	1.60 de	2.30 b
Plant oils recommended in plant protection				
Dedal 90 EC	1.0	0.55 e	1.45 bc	3.00 de
Olejan 80 EC	1.0	0.50 de	1.35 b	2.90 cd
Mineral oils				
Atpolan 80 EC	1.0	0.30 b	1.60 de	2.80 c
Ikar 95 EC	1.0	0.35 bc	1.50 cd	2.80 c
Olemix 84 EC	1.0	0.40 b–d	1.70 ef	3.15 e
Promanal 60 EC	1.0	0.55 e	1.80 f	3.15 e

Note: Mean values marked with the same letter in each observation do not differ at the significance level p = 0.05 according to the Duncan's test.
Disease index: 0 – no diseases symptoms, 1 – from 0.1 tp 25 % of leaves with disease symptoms, 2 – over 25 % of leaves with disease symptoms, 3 – up to 25 % of fallen leaves and the rest with disease symptoms, 4 – from 25 to 50 % of fallen leaves, 5 – from 50 to 90 % of fallen leaves, 6 – over 90 % of fallen leaves.

Table 3

Effectiveness of some oils in the control of *Diplocarpon rosae* Wolf.
on roses cv. Red Berlin. Mean degree of shoots infection.
Beginning of experiment and initial disease level: 2005.06.15 = 0.3

Treatment	Conc. in %	After sprayings		
		3	6	9
Control	—	1.35 f	3.10 h	5.80 f
Saprol 190 EC	0.15	0.25 a	0.70 a	1.45 a
Score 250 EC	0.05	0.15 a	0.60 a	1.45 a
Plant oils used as a food (Cooking oil)				
Corn oil (maize oil)	1.0	0.80 de	1.90 fg	2.40 b
Grape seed oil	1.0	0.80 de	2.00 g	2.70 d

Table 3 contd.

Treatment	Conc. in %	After sprayings		
		3	6	9
Olive oil	1.0	0.70 de	1.55 bc	2.40 b
Rape oil (canola oil)	1.0	0.50 b	1.55 bc	2.75 de
Soybean oil	1.0	0.75 de	1.70 c–e	2.80 de
Sunflower oil	1.0	0.55 bc	1.75 d–f	2.70 d
Plant oils recommended in plant protection				
Dedal 90 EC	1.0	0.75 de	1.60 b–d	2.70 d
Olejan 85 EC	1.0	0.75 de	1.50 b	2.70 d
Mineral oils				
Atpolan 80 EC	1.0	0.80 e	1.55 bc	2.80 de
Ikar 95 EC	1.0	0.85 e	1.85 e–g	2.55 c
Olemix 84 EC	1.0	0.75 de	1.85 e–g	2.85 e
Promanal 60 EC	1.0	0.85 e	1.70 c–e	2.80 de

Explanation – see Table 1.

Table 4

Effectiveness of some oils in the control of *Diplocarpon rosae* Wolf.
on roses cv. Red Berlin. Mean degree of shoots infection.
Beginning of experiment and initial disease level: 2006.05.30 = 0.45

Treatment	Conc. in %	After sprayings		
		3	6	9
Control	—	2.18 e	4.03 g	5.53 i
Control + water	—	2.30 e	4.20 h	5.70 j
Score 250 EC	0.05	0.60 a	1.40 a	2.75 a
Plant oils used as a food (Cooking oil)				
Corn oil	1.0	1.25 bc	2.70 e	3.90 gh
Olive oil	1.0	1.30 cd	2.95 f	3.75 d–f
Rape oil (canola oil)	1.0	1.30 cd	2.60 de	3.65 b–d
Soybean oil	1.0	1.30 cd	2.85 f	3.90 gh
Sunflower oil	1.0	1.40 d	2.85 f	3.55 b
Plant oils recommended in plant protection				
Dedal 90 EC	1.0	1.30 cd	2.35 b	3.70 c–e
Olejan 85 EC	1.0	1.20 bc	2.45 bc	3.60 bc
Mineral oils				
Atpolan 80 EC	1.0	1.15 b	2.45 bc	3.80 e–g
Ikar 95 EC	1.0	1.20 bc	2.45 bc	3.85 f–h
Olemix 84 EC	1.0	1.30 cd	2.40 bc	3.90 gh
Promanal 60 EC	1.0	1.20 bc	2.45 bc	3.80 e–g
Sunspray 840 EC	1.0	1.20 bc	2.50 cd	3.95 h

Explanation – see Table 1.

In 2006, after the 3rd spray treatment of the roses, the percentage effectiveness of the tested oils ranged from 35.8 % (sunflower oil) to 47.2 % (Atpolan 80 EC). After the 6th treatment, the effectiveness of the tested oils decreased slightly and ranged from 26.8 % (olive oil) to 41.7 % (Dedal 90 EC). After spraying the plants 9 times, the effectiveness of the tested oils relative to the control roses remained at a similar level and ranged from 29.5 % (corn oil and Olemix 84 EC) to 46.6 % (Sunspray 840 EC). After the 3rd treatment, the severity of infection of the shrubs was below 2.0. However, after 6 treatments, the severity of infection fluctuated already around 4.0, which was associated with severe dropping of leaves and a drastic decrease in the decorative value of the shrubs. The tested vegetable and mineral oils were significantly less effective in comparison with the fungicide Saprol 190 EC (Table 4).

Discussion

The results of the experiments do not make it possible to state clearly which of the tested vegetable or mineral oils showed the highest effectiveness in inhibiting the development of disease symptoms and the pathogen. The percentage effectiveness of the tested oils relative to the control depended on the oil being tested, the time of observation and the experiment. Our results contrast, therefore, with those of Vawdrey et al. [6], who, while conducting field experiments on controlling yellow Sigatoka of banana, demonstrated superior effectiveness of mineral oils over vegetable oils.

As the severity of disease symptoms during the growing season increased, the effectiveness of the tested oils decreased. It seems that during the initial period of vegetation, even on very susceptible varieties, the effectiveness of the tested oils was sufficiently high until the end of June. However, in the second half of July and in August, when the weather conditions changed (cool nights, dew), the development of *D. rosae* proceeded more quickly and the effectiveness of the oils decreased significantly. It should be emphasized that in the author's own experiments, the same oils used for spraying roses showed a significant inhibiting effect on the germination of *D. rosae* spores, and thus on inhibiting the development of disease symptoms. In addition, the deformation of spores caused by the application of the oils, which could be seen under the scanning electron microscope, did not explicitly translate into the inhibition of the germination of spores [34].

In the available literature, data on the effectiveness of oils in controlling *Diplocarpon rosae* on roses are very scarce. Horst et al. [9] showed that powdery mildew, caused by *Sphaerotheca pannosa* (Wallr. Ex Fr.) Lév. var. *rosae* Wor., and black spot, caused by *Diplocarpon rosae*, were significantly controlled by weekly sprays of 0.063 M aqueous solution of sodium bicarbonate plus 1.0 % (v/v) Sunspray ultrafine spray oil on *Rose* spp. Experiments by Osnaya-Gonzales et al [35] also confirmed the high effectiveness of oils in a mixture with sodium bicarbonate in controlling black spot. The authors demonstrated that formulated rapeseed oil (Telmion®) or neem oil in combination with $NaHCO_3$ gave more than 90 % effectiveness in the control of *Diplocarpon rosae*, the causal agent of black spot of rose. In their subsequent experiments, the authors also found that all vegetable oils (rapeseed, coconut, maize, olive, safflower, sesame, soybean, sunflower) in combination with $NaHCO_3$ affected the development of black spot on detached leaflets of *Rosa canina pollmeriana*, when applied as protective treatments. The necrotic lesions were smaller and the number of acervuli/lesions lower. In both experiments, maize and soybean oils were inferior to all the other oils [11]. Also, a significant increase in the fungicidal activity of potassium bicarbonate against apple scab (*Venturia inaequalis* (Cooke) Winter) was observed when salt was mixed with mineral oil. However, combining potassium bicarbonate with vegetable linseed oil and grapefruit seed extract did not increase its efficacy, whereas these two vegetable products used alone significantly reduced scab infections [10]. Northover and Schneider [15] demonstrated that sunflower, olive, canola, corn, soybean, and grapeseed oils showed only slight prophylactic activity against *Venturia inaequalis* under controlled conditions. A total of 10 applications of canola or soybean oil emulsified with Agral 90 and applied under orchard conditions reduced foliar and fruit infection with *V. inaequalis* by 66 and 81 %, respectively.

In view of the safety of vegetable oils to the environment and their almost 100 % effectiveness in controlling *S. pannosa* var. *rosae*, the main disease of roses, the possibility of inhibiting the development of *Botrytis cinerea* Pers., the causal agent of grey mould, and of *Phragmidium mucronatum* (Pers.) Schlecht. (although in other species of plants) [17, 36], these oils should certainly be included in the programme for the protection of roses from disease. It should be emphasized that the experiments on the effectiveness of the oils was carried out on a rose variety that is very susceptible to black spot. In practice, one can observe a tendency for planting those varieties that are low-susceptible or resistant to disease. In case of a need to protect low-susceptible varieties, the effectiveness of the tested oils will certainly be sufficient.

The results of the experiments have clearly shown that oils can play an important role as an alternative to synthetic fungicides in the protection of roses against black spot.

Conclusions

1. The obtained results do not make it possible to draw a clear conclusion as to which of the tested vegetable or mineral oils showed the highest effectiveness in inhibiting the development of the symptoms of black spot and the fungus *D. rosae*.

2. Depending on the experiment and the time of observation, the percentage inhibition of the development of disease symptoms was found to vary. After 3 spray treatments, the effectiveness of the tested oils varied between 35.8 % (sunflower oil) and 77.8 % (Atpolan 80 EC); after 6 applications, it was 26.8 % (olive oil) – 55 % (Olejan 85 EC); and after 9 applications, 29.5 % (corn oil, soybean oil and Olemix 84 EC) – 58.6 (olive oil).

3. There was no evidence of phytotoxicity of the tested oils to the rose plants.

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WPŁYW OLEI ROŚLINNYCH I MINERALNYCH NA ROZWÓJ *Diplocarpon rosae* Wolf. SPRAWCĘ CZARNEJ PLAMISTOŚCI RÓŻY

Zakład Ochrony Roślin Ozdobnych
Instytut Ogrodnictwa w Skierniewicach

Abstrakt: W przeprowadzonych badaniach polowych określano wpływ olei roślinnych (kukurydziany, olej z oliwek, rzepakowy, słonecznikowy, sojowy, winogronowy) lub olei roślinnych polecanych jako adjuwanty: Dedal 90 EC (90 % vegetable oil), Olejan 80 EC (85 % rape oil) oraz olei mineralnych Atpolan 80 EC (76 % SN oil), Ikar 95 EC (95 % SAE petroleum oil), Olemix 84 EC (84 % DSA petroleum oil), Promanal 60 EC

(60 % petroleum oil) stosowanych w stęż. 1 % do 9-krotnego opryskiwania krzewów róż w odstępach 7-dniowych w zwalczaniu czarnej plamistości powodowanej przez *Diplocarpon rosae*.

Uzyskane wyniki nie pozwalają na wyciągnięcie jednoznacznego wniosku, który z badanych olei roślinnych lub mineralnych wykazywał najwyższą skuteczność w ograniczaniu rozwoju objawów czarnej plamistości oraz grzyba *D. rosae*. Stwierdzono, że procentowa skuteczność badanych olei względem kontroli była uzależniona od rodzaju oleju, terminu obserwacji oraz doświadczenia. W zależności od doświadczenia i terminu obserwacji stwierdzono zróżnicowane procentowe ograniczanie rozwoju objawów chorobowych. Po 3-krotnym opryskiwaniu róż skuteczność badanych olei wahała się od 35,8 % (sunflower oil) do 77,8 % (Atpolan 80 EC), po 6-krotnym opryskiwaniu od 26,8 % (olive oil) do 55 % (Olejan 85 EC) oraz po 9-krotnym opryskiwaniu od 29,5 % (corn oil, soybean oil and Olemix 84 EC) do 58,6 % (olive oil). Nie stwierdzono fitotoksyczności badanych olei w stosunku do odmiany róż, na której prowadzono doświadczenia.

Słowa kluczowe: *Diplocarpon rosae*, oleje roślinne i mineralne, zwalczanie, róża

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Katarzyna SOŁEK-PODWIKA[1], Miłosz PODWIKA[2]
and Joanna NIEMYSKA-ŁUKASZUK[1]

TRACE ELEMENT CONCENTRATION IN SOIL OF SELECTED FORESTS OF KRAKOW CITY

ZAWARTOŚĆ PIERWIASTKÓW ŚLADOWYCH W GLEBACH WYBRANYCH LASÓW MIASTA KRAKOWA

Abstract: The aim of the paper was to evaluate the total content of cadmium, lead and chromium in the humus horizons of the forest and turf soils, rendzinas and mucky soils, located in the city of Krakow. In the examined soils; rendzinas and mucky soil, located in the south-western part of Krakow, the accumulation of these elements did not exceed limits considered as harmful. In the humus horizons of the examinated soils the anthropogenic accumulation of Cd and Pb which was estimated and confirmed by the calculated coefficient of accumulation. The accumulation coefficient was highier for mucky soils than for rendzinas.

Keywords: trace elements, turf and forest soils, the city of Krakow.

Introduction

The amount of heavy metals found in soil depends on many factors, both natural and anthropogenic [1]. In the current changes of the natural environment we can often observe the domination of multidirectional economic activity of man over natural causes, creating soil forming processes [2, 3]. Under the influence of gas emissions and the liquids from industrial institutions as well as the different elements of traffic pollution different types of chemical elements get into soil and accumulate there.

The aim of this research was to determine the influence of human activity and pollution on the total content of cadmium, lead and chromium in the humus horizons of soil differently used (forest and turf), representing two types of soil, located in the area of Krakow.

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[1] Soil Science and Soil Protection Department, Agricultural University in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 43 70; fax: +48 12 633 62 45; email: rrpodwik@cyf-kr.edu.pl

[2] Local Parc and Zoological Garden Foundation in Krakow, ul. Kasy Oszczędności Miasta Krakowa 14, 30–232 Kraków, Poland, phone: +48 12 425 35 51; fax: +48 12 424 27 10; email:miloszp@interia.pl

Materials and the methods of investigation

The area chosen for the research was located in the south-western part of the city of Krakow. The main risks in the area of research which have an anthropogenic character are mainly caused by pollution connected with car transport and communication. The object of research were forest and turf soils which, according to the IV issue of Polish Soil Systematics (1989), belong to the mucky soils and mixed rendzinas, with loamy light and heavy sand texture. The comparison of the content of cadmium, lead and chrome in the humus horizons with a depth of 0–20 cm, became possible thanks to the realization of turf soil profiles neighbouring the profiles of forest soils. At the same time the studied soils were found in the same physiographic conditions (with similar configuration of terrain, climate, similar water relations and the origin of geological parent rocks), these are soils with similar soil texture. This way of selecting the soil made it possible to determine the influence of economic activity of man, because the main factor differentiating the proprieties of the tested soil types was their use.

In the collected soil material the total content of cadmium, lead and chrome was determined in air dried samples using atomic absorption spectrometry (AAS) after soil dissolving in a mixture of concentrated nitric(V) and chloric(VII) acids. For the studied humus horizons *coefficients of accumulation* (AC) were counted for cadmium and lead (in relation to their content in the parent rock). In order to determine the properties of some physicochemical properties of the studied soils the following analyses were performed: soil texture according to the areometric Casagrande method in modification by Proszynski, pH by the potentiometer in 1 mol KCl $\cdot$ dm^{-3}, organic C concentration by oxidation with potassium dichromate(VI) using the modified Tiurin method.

Results and discussion

Table 1 presents the results of the performed analyses of the soil reaction, the content of organic carbon and elements of cadmium, lead, and chromium. Both humus horizons of forest and turf soils of the studied types of soil were characterized by the approximate pH values. Rendzinas were characterized by a neutral reaction through weak acid, acid to strong acid, and mucky soils from acid to strong acid. Forest soils of both types were generally richer in organic carbon than turf soils (Table 1).

According to statistical analyses and the use of the t-Student test it was confirmed, that the content of the chosen elements (Cd, Pb and Cr) was not connected with content of organic carbon, the soil reaction and the granulation of studied soils. The similar lack of dependence between proprieties of soils and the content of Cu, Ni and Zn was also confirmed by Mazurek and others [4] researching the content of these elements in forest soils located in the city of Krakow.

The mineral parent rock materials of the studied soils, ie rendzinas and mucky soils, respectively were characterized by the following content of trace elements: the Jurassic limestone 0.54 mg Cd $\cdot$ kg^{-1} and 54.17 mg Pb $\cdot$ kg^{-1} and fluvioglacial sands 0.1 mg Cd $\cdot$ kg^{-1} and 4.47 mg Pb $\cdot$ kg^{-1}. The content of cadmium and lead in fluvioglacial sand is smaller than that quoted by Czarnowska [5] for the geochemical background of different origins of sand from northern and central Poland.

Table 1

Total content of cadmium, lead and chrome and selected soil properties of studies soils

No. profile	pH in KCl	C_{org} [g · kg^{-1} soil]	Cd [mg · kg^{-1} soil]	AC	Pb [mg · kg^{-1} soil]	AC	Cr [mg · kg^{-1} soil]
RENDZINAS							
Turf soils							
1	4.1	14.9	1.05	1.9	34.09	0.6	7.08
2	6.9	34.1	0.38	0.7	39.47	0.7	13.75
Forest soils							
3	6.5	11.8	1.97	3.7	76.74	1.4	21.97
4	5.0	54.9	1.98	3.7	89.82	1.7	23.70
5	6.8	59.4	1.02	1.9	32.80	0.6	9.07
6	4.1	52.4	1.19	2.2	59.60	1.1	16.22
7	4.7	62.3	2.03	3.8	82.27	1.5	15.40
MUCKY SOILS							
Turf soils							
8	4.4	31.3	0.91	9.1	20.82	4.7	3.82
9	5.0	19.8	2.08	20.8	78.48	17.6	18.97
Forest soils							
10	3.7	44.2	0.59	5.9	23.47	5.3	10.25
11	4.0	11.5	0.86	8.6	29.18	6.5	11.22
12	5.0	33.4	1.19	11.9	61.19	13.7	6.89
13	4.9	33.2	0.76	7.6	20.19	4.5	11.22

The value of *coefficients of accumulation* (AC) for cadmium and lead calculated for the humus horizons of the studied soils in the majority of cases confirm the anthropogenic origin of the accumulated metals. Higher accumulation coefficients for Cd, which varied in the range from 5.9 to 20.80 and Pb from 4.52 to 17.56, were calculated for mucky soils than rendzinas (Table 1). High values of coefficients of enrichment according to many authors [1, 6], result in small amounts of these metals in parent rocks.

The total content of cadmium in the horizons of rendzins covered with forest varied between 1.02 to 2.03 mg · kg^{-1} the soil and was about twice higher than in analogous horizons of turf soils (0.38–1.05 mg · kg^{-1} soil), in which also generally smaller total content of lead and chromium were found. The opposite dependence for studied heavy metals was estimated in mucky soils, in which the total content of cadmium (0.59–1.19 mg · kg^{-1} soil) in the humus horizons of forest soils was smaller than in analogous horizons of turf soils (0.91–2.08 mg · kg^{-1} soil). The total content of lead and chromium in mucky soils of different use are shaped similarly, they are generally larger in horizons of turf soils than forest ones. On the basis of the results of the conducted research we cannot unambiguously determine a connection between the studied elements and the different way of use of the studied soils. The similar lack of relation in the total content of different trace elements between forest soils and the cultivated ones were affirmed by Kocowicz [7] as well as Zablocki and others [8].

Anthropogenic intensification can be estimated through the comparison of the amount of the studied metals with their admissible values. At present the estimation of the state of soils is mostly carried out with the support of the Decree of Minister of the Environment on the basis of soil quality standards as well as the standards of quality of the ground dated 9th September 2002 [9], this act is the legal basis for the protection of the environment, this act defines in section IV the principles of soil protection. This Decree establishes the admissible values of contaminants in the soil and ground for different substances, included heavy metals, in three groups of grounds and soils. In the studied soils, which were placed in group B, including among others different agricultural soils, forest soils, waste lands: (depth 0.0–0.3 m under ground level) according to the Decree of the Minister of the Environment [9] the influence of city and industrial agglomeration in Krakow did not confirm exceeding the admissible values of concentrations of selected trace elements.

Conclusion

1. In the humus horizons of the studied soils on the basis of the calculated coefficients of enrichment, the anthropogenic accumulation of cadmium and lead was confirmed, higher for mucky soils than for rendzinas
2. On the basis of the results we cannot unambiguously determine a connection between the total content of the studied elements in the studied soils of different use.
3. The studied soils which were located in the south-western part of the city of Krakow can be ccounted as areas where the concentration of the chosen trace elements does not exceed admissible amounts defined in the Decree of the Minister of the Environment.

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ZAWARTOŚĆ PIERWIASTKÓW ŚLADOWYCH W GLEBACH WYBRANYCH LASÓW MIASTA KRAKOWA

[1] Katedra Gleboznawstwa i Ochrony Gleb
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
[2] Fundacja Miejski Park i Ogród Zoologiczny w Krakowie

Abstrakt: Celem przedstawionych badań było określenie całkowitej zawartości kadmu, ołowiu i chromu w poziomach próchnicznych gleb leśnych i zadarnionych; rędzin i gleb murszowatych położonych na terenie miasta Krakowa. Badane gleby położone w południowo-zachodniej części miasta Krakowa zaliczono do obszarów, gdzie koncentracja wybranych pierwiastków śladowych nie przekraczała norm uważanych za szkodliwe. W poziomach próchnicznych badanych gleb stwierdzono antropogenne nagromadzenie Cd i Pb co potwierdziły obliczone współczynniki wzbogacenia, które były większe dla gleb murszowatych niż rędzin.

Słowa kluczowe: pierwiastki śladowe, gleby zadarnione i leśne, miasto Kraków

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Bożena DĘBSKA[1], Dorota KALEMBASA[2]
and Sławomir GONET[3]

PARAMETERS OF ORGANIC MATTER OF SOIL IN THE CULTIVATION OF *Miscanthus sacchariflorus*

PARAMETRY MATERII ORGANICZNEJ GLEBY POD UPRAWĄ TRAWY *Miscanthus sacchariflorus*

Abstract: Selected properties of organic matter in the humus horizon of soil fertilised with sludge at 10, 20 and 30 Mg · ha^{-1} and with mineral fertilisers (NPK), were examined in the third year after harvesting biomass of *Miscanthus sacchariflorus*. The following were analysed: TOC, N_t in the soil; the following were analysed in the humic acids isolated from samples – elemental composition, atomic ratios, degree of internal oxidation, absorption coefficient, hydrophilic and hydrophobic properties, IR and ^{13}C NMR spectra (the latter two were used for determination of carbon species). The organic carbon content was found to increase in the pot fertilised with the largest dose of the sludge fertiliser (30 Mg · ha^{-1}), while the nitrogen content increased in all the pots fertilised with the organic waste material, as compared with the control pot. Humic acids, isolated from the pots fertilised with sludge and mineral fertilisers (NPK), were found to have a higher degree of internal oxidation. The variance of the other quality parameters of those acids was not significant. Fertilisation with sludge did not significantly affect the properties of humic acids in the third year of cultivation of the test grass, which is important regarding the balance of the soil.

Keywords: *Miscanthus sacchariflorus*, sewage sludge, organic matter, infrared (IR) spectra, HPLC, ^{13}C NMR

Introduction

The growing demand for energy, with its increasingly restricted supply, has been encouraging scholars to seek alternative sources. New energy sources are increasingly often sought among agricultural products. Plant species cultivated as material providing energy should be highly capable of fixing solar energy and transforming it to organic matter. They should also be capable of taking up nutrients from soil and their growth rate should be high. It is necessary to be able to obtain a high yield of biomass at a low

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[1] Department of Environmental Chemistry, University of Technology and Life Sciences, ul. Bernardyńska 6, 85–029 Bydgoszcz, Poland, email: debska@utp.edu.pl

[2] Soil Science and Plant Nutrition Department, Siedlce University of Natural Sciences and Humanities, ul. B. Prusa 14, 08–100 Siedlce, Poland, email: kalembasa@uph.edu.pl

[3] Department of Soil Science, Nicolaus Copernicus University, ul. Gagarina 9, 87–100 Toruń, Poland, email: gonet@geo.uni.torun.pl

cost of cultivation. Therefore, it is important to select an appropriate plant, suitable for a local habitat (soil, climate).

Among the plants cultivated as energy sources are grasses of genus *Miscanthus*, whose features – according to Hryniewicz and Grzybek [1] – include low energy consumption. The plants have thick, rigid stems filled with spongy cores and contain large amounts of lignin and cellulose. *Miscanthus* grasses can be used for 20 years, with most of financial outlays being made during the first year. It is an advantage of some of its species, *eg Miscanthus sacchariflorus*, that it can be cultivated on soils fertilised with sludge [2]. Using sludge for fertilisation of energy plants enables one to achieve higher biomass yield and better physicochemical soil properties [3].

The aim of the study was to evaluate the effect of NPK fertilisation of varied rates of fertilisation with sludge of *Miscanthus sacchariflorus* on the content of organic carbon and nitrogen in soil and on the properties of humic acids.

Material and methods

Samples taken from the humus horizon of the soil used in the cultivation of *Miscanthus sacchariflorus,* in the third year after biomass harvest, from plots with varied fertilisation options: no fertilisation – control (sample marked as "a"); mineral fertilisation (NPK) with the element ratio of 1 : 0.8 : 1.2 ("b"); 10^4 kg · ha^{-1} of dry matter of sludge ("c"); $2 \cdot 10^4$ kg · ha^{-1} of dry matter of sludge ("d"); $3 \cdot 10^4$ kg · ha^{-1} of dry matter of sludge ("e"), were used in the study.

The sludge is characterised in Table 1 [2]. The amount of nitrogen introduced to soil with the NPK fertilisers was equivalent to the amount of nitrogen in $2 \cdot 10^4$ kg · ha^{-1} of dry matter of sludge.

Table 1

Chemical composition of waste activated sludge [2]

Dry matter [%]	pH	TOC	N	TOC : N	P	K
		[g · kg^{-1}]	[g · kg^{-1} d.m.]			
24.5	6.5	351	41.50	8.5 : 1	20.78	2.90

The following were determined in the samples total organic carbon (TOC) and total nitrogen (N_t) – with the VarioMax CN analyzer by Elementar (Germany). *Humic acids* (HAs) were extracted and purified according to standard methods using the following procedure:

– decalcification with 0.05 M HCl at 1 : 10 (w/v) ratio, extraction time 24 h. After centrifugation of the solution, the residue was washed with distilled water until a neutral reaction was reached;

– extraction of humic acids: solid remaining after decalcification was subjected to 0.5 M NaOH at 1 : 10 (w/v), with 24 h extraction time, occasionally mixed, then centrifuged humic acids extract;

– precipitation of humic acids: the alkaline extract obtained was treated with 2 M HCl giving pH = 2, after 24 hours the precipitate of humic acids was separated by centrifugation;

– purification of humic acids, the residue of humic acids obtained was treated with a mixture of HCl-HF (990 cm^3 H_2O, 5 cm^3 HCl and 5 cm^3 HF) over 24 hours and subsequently separated in a centrifuge. This action was repeated three times. After purification the residue of humic acids was treated with distilled water until a zero reaction to chlorides was achieved, then freeze-dried.

The extracted humic acids were analyzed for:

a) elemental composition (Perkin Elmer CHN analyzer, Series II 2400). On the basis of elemental composition the values of atomic ratios: H/C, O/C, O/H, N/C and ω – internal oxidation degree were calculated according to the formula:

$$\omega = (2O + 3N\text{-}H) : C,$$

where: O, N, H, C – content in atomic percentage;

b) absorption spectra in UV-VIS range (Perkin Elmer UV-VIS Spectrometer, Lambda 20). VIS spectra were performed for 0.02 % humic acids solutions in 0.1 M NaOH and UV-spectra were determined after fivefold dilution. Absorbance measured at the wavelengths 280 nm (A_{280}), 400 nm (A_{400}), 465 nm (A_{465}), 600 nm (A_{600}) and 665 nm (A_{665}) were used to calculate coefficient values:

– $A_{2/4}$ – 280 nm and 465 nm absorbance ratio,

– $A_{2/6}$ – 280 nm and 665 nm absorbance ratio,

– $A_{4/6}$ – 465 nm and 665 nm absorbance ratio,

– $\Delta\log K = \log A_{400} - \log A_{600}$;

c) infrared spectra (Perkin-Elmer FT-IR Spectrometer, Spectrum BX). FT-IR spectra for the range 400–4400 cm^{-1} were done for HA samples of 3 mg in 800 mg KBr. To carry out IR spectra processing, the deconvolution method was applied, with a filter making the bands of $\gamma = 4$ narrower, and the process of smoothing, for which the length parameter was l = 80 % [4];

d) ^{13}C NMR spectra – with the spectrometer MSL-300 by Bruker. Based on the areas defined under peaks [5, 6], there was calculated the share of carbon in the following bonds:

– C_{al} – carbon in aliphatic bonds (0–108 ppm),

– C_{ar} – carbon of aromatic bonds (108–145 ppm),

– C_{lig} – carbon of aromatic lignin bonds (145–165 ppm),

– C-CO – carbon of carboxyl groups (165–180 ppm) and carbonyl groups (180–200 ppm) and parameters:

– C_{al}/C_{ar} – ratio of carbon of aliphatic bonds (C_{al}) to carbon of aromatic bonds and lignin bonds ($C_{ar} + C_{lig}$);

e) hydrophilic and hydrophobic properties with the use of liquid chromatograph HPLC Series 200 with DAD detector (Perkin-Elmer). The separation involved the use of column X-Terra C18, 5 μm, 250 · 4.6 mm. Solutions of humic acids were applied in 0.01 mol/dm^3 NaOH of the concentration of 2 mg/cm^3; injection of the sample – 0.1 cm^3 ; solvent–acetonitril–water; solvents flow in the gradient (ratio H_2O: ACN (v/v) over 0–6 min – 99.5 : 0.5, 7–13 min – 70 : 30, 13–20 min – 10 : 90); detection – at the wavelength of 254 nm. Based on the areas determined under peaks, the share of *hydrophilic* (HIL) and *hydrophobi*c (HOB = HOB-1 + HOB-2) fractions in humic acids molecules and the parameter: HIL/HOB [4, 7, 8].

Results and discussion

The TOC content in the soil samples ranged from 28.47 (plot fertilised with NPK) to 32.25 g · kg^{-1} (plot with 3 · 10^4 · ha^{-1} of sludge) (Table 2). It ranged from 29.61 to 30.64 g · kg^{-1} in the soil of the other plots, regardless of fertilisation option. The nitrogen content (N_t) in the soil of the plots where no sludge was added, was similar – *ca* 1.5 g · kg^{-1}. The addition of sludge to soil increased the content of N_t by 19 to 45 %, as compared with soil with no fertilisation; the largest growth was caused by addition of 30 Mg · ha^{-1} of sludge. An increase in the amount of N_t in soil fertilised with sludge reduced the TOC/N_t ratio, as compared with soil with no fertilisation (control) and that fertilised with NPK.

Table 2

TOC and N_t content and ratio values of TOC/N_t in the analyzed soil samples

Object	TOC	N_t	TOC /N_t
	[g · kg^{-1}]		
Control	30.64	1.52	20.16
NPK	28.47	1.51	18.85
10 Mg · ha^{-1} of the sludge*	30.78	1.95	15.78
20 Mg · ha^{-1} of the sludge	29.61	1.81	16.36
30 Mg · ha^{-1} of the sludge	32.25	2.21	14.59

* – waste activated sludge.

Humic acids are one of the most important fractions of organic matter; they contribute to physical, biological and chemical properties of soil and immobilise toxic compounds, *etc* [4, 9].

The main elements which comprise *humic acids* (HAs) include carbon, hydrogen, oxygen and nitrogen. The elemental composition of humic acids, expressed as weight and atom percent, is shown in Table 3.

Table 3

Elemental composition of humic acids

Object	C	H	N	O	C	H	N	O
	weight [%]				atomic [%]			
Control	51.49	5.34	4.30	38.87	34.70	43.17	2.48	19.65
NPK	52.10	5.19	4.17	38.54	35.48	42.41	2.43	19.68
10 Mg · ha^{-1} of the sludge*	52.35	5.17	4.24	38.24	35.68	42.29	2.48	19.55
20 Mg · ha^{-1} of the sludge	51.46	5.22	4.20	39.12	34.98	42.62	2.44	19.95
30 Mg · ha^{-1} of the sludge	51.43	5.26	4.39	38.91	34.86	42.82	2.55	19.78

* – waste activated sludge.

Humic acids in soil fertilised with larger doses of sludge (20 and 30 Mg · ha^{-1}) contained smaller amounts of carbon as compared with HAs in soil fertilised with NPK and sludge at 10 Mg · ha^{-1}. The hydrogen content in HA molecules in the pots fertilised with sludge and NPK was lower than or close to (30 Mg · ha^{-1} of sludge), as compared with humic acids in soil where no fertilisation was applied. The nitrogen and oxygen content in HAs of the soil did not differ much, regardless of the fertilisation option. A slightly higher content of oxygen was found in HAs in soil fertilised with sludge at 20 Mg · ha^{-1}. The H/C ratio lay within the range from 1.19 to 1.24 (Table 4). No significant differences in the N/C, O/C or O/H values were found. Soil fertilisation with NPK and sludge at 10 to 30 Mg · ha^{-1} increased the degree of internal oxidation in HA molecules. The lowest value of the parameter was determined for HAs in soil with no fertilisation.

Table 4

Value of atomic ratios ω and degree of internal oxidation of humic acids

Object	H/C	N/C	O/C	O/H	ω
Control	1.24	0.072	0.57	0.46	0.103
NPK	1.20	0.069	0.55	0.46	0.120
10 Mg · ha^{-1} of the sludge*	1.19	0.069	0.55	0.46	0.119
20 Mg · ha^{-1} of the sludge	1.22	0.070	0.57	0.47	0.132
30 Mg · ha^{-1} of the sludge	1.23	0.073	0.57	0.46	0.126

* – waste activated sludge.

The value of absorbance at the wavelengths of λ = 280, 400, 465, 600 and 665 nm was used to determine the absorbance ratios $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and ΔlogK (Table 5). The $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and ΔlogK coefficients can be used to evaluate the advancement of humification of organic matter and to characterise the resultant humic substances [4, 10, 11]. The absorbance values for humic acids isolated from soil taken from plots where fertilisers were applied were usually higher as compared with the control. The absorbance coefficients $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and ΔlogK HAs for soil fertilised with NPK and sludge at 10 and 20 Mg · ha^{-1} were lower compared with the soil with no fertilisation applied and soil fertilised with sludge at 30 Mg · ha^{-1}.

Table 5

Value of absorbance at the wavelengths of humic acids

Object	A_{280}	A_{400}	A_{465}	A_{665}	$A_{2/4}$	$A_{2/6}$	$A_{4/6}$	ΔlogK
Control	2.93	1.21	0.626	0.104	4.68	28.23	6.03	0.790
NPK	3.00	1.28	0.667	0.113	4.50	26.47	5.89	0.777
10 Mg · ha^{-1} of the sludge*	2.94	1.26	0.656	0.111	4.48	26.54	5.93	0.782
20 Mg · ha^{-1} of the sludge	2.87	1.22	0.633	0.110	4.53	26.10	5.76	0.780
30 Mg · ha^{-1} of the sludge	2.81	1.21	0.621	0.102	4.52	27.54	6.09	0.796

* – waste activated sludge.

In the molecules of the humic acids analyzed there were isolated hydrophilic (HIL) and hydrophobic fractions (HOB-1 and HOB-2). Peaks found on chromatograms (Fig. 1) ranging from 2.4 to 7.5 min. correspond to hydrophilic fractions, while peaks ranging from 17.0 to 25.0 min. correspond to hydrophobic fractions (HOB) [4, 7, 8].

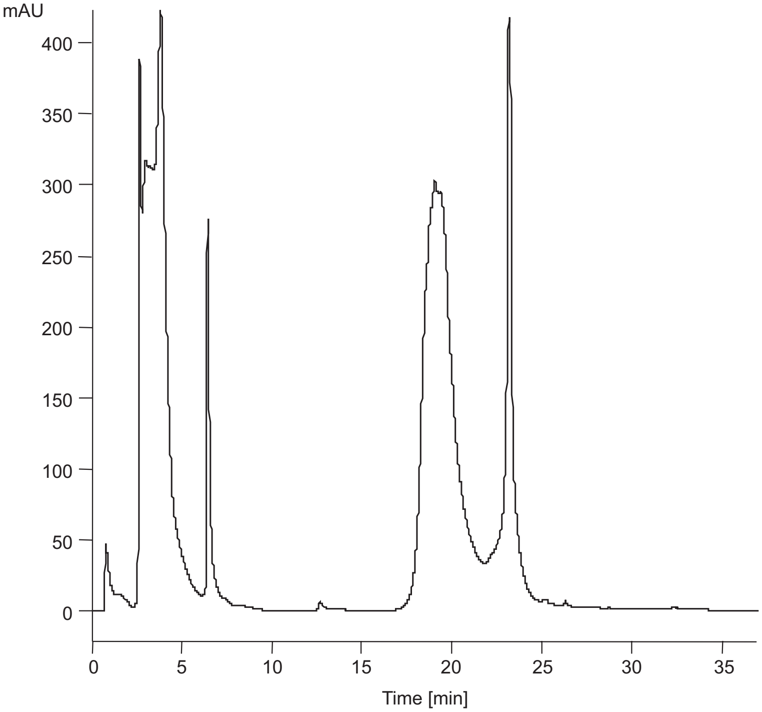

Fig. 1. Example of the chromatogram (HPLC) of humic acids (plot fertilised with 20 Mg · ha^{-1} of sludge)

Fraction HOB-1 is a hydrophobic fraction of a shorter retention time from 17.2 to 21.0 and fraction HOB-2 ranges from 21.0 to 25.0 min. The share of hydrophobic fractions considerably exceeded the share of hydrophilic fractions (Table 6).

Table 6

Parameters describing the hydrophilic and hydrophobic properties of humic acids

Object	HIL-1	HIL-2	Σ HIL	HOB-1	HOB-2	Σ HOB	HIL/HOB
Control	42.3	4.5	46.8	38.6	14.6	53.2	0.878
NPK	41.5	4.6	46.1	39.5	14.4	53.9	0.854
10 Mg · ha^{-1} of the sludge*	40.7	4.6	45.3	40.6	14.1	54.7	0.829
20 Mg · ha^{-1} of the sludge	41.5	4.5	46.0	39.3	14.7	54.0	0.852
30 Mg · ha^{-1} of the sludge	41.2	4.7	45.9	39.5	14.6	54.1	0.850

* – waste activated sludge.

The application of NPK fertilisers and varied rates of fertilisation with sludge slightly modified hydrophilic and hydrophobic properties of analyzed humic acids. The HAs of objects fertilised with sludge and mineral fertilisers characterized by slightly lower contribution of hydrophilic fractions and higher contribution of hydrophobic ones, compared with the not fertilised soil.

Above relationships indicate slight effect of type of fertilisation on the properties and structure of humic acids (in the third year after application), which is confirmed by ^{13}C NMR and IR spectra.

In the spectra of the HAs (Fig. 2), there was identified the presence of the band in the range of wavenumbers 3100–3600 cm^{-1} corresponding to the stretching vibration of –OH groups in alcohols, phenols and acids and NH. The inflection noted in the range of wavenumbers 3000–3100 cm^{-1} is connected with the presence of CH bonds (valence vibration) of aromatic and alicyclic compounds. The bands in the 2920–2960 cm^{-1} range and around 2850 cm^{-1} are conditioned by the presence of groups $-CH_3$ and $=CH_2$ (symmetric and antisymmetric stretching vibration). The 1710–1730 cm^{-1} band points to the presence of carbonyl groups C=O (stretching vibration) in the acids and ketones and the bands in the range of wavenumbers 1600–1660 cm^{-1} are connected with the occurrence of stretching vibration C=O (1630–1680 cm^{-1}), NH deformation vibration (1620–1660 cm^{-1}) in the primary amides, and C=C stretching vibration (1610 cm^{-1}) in

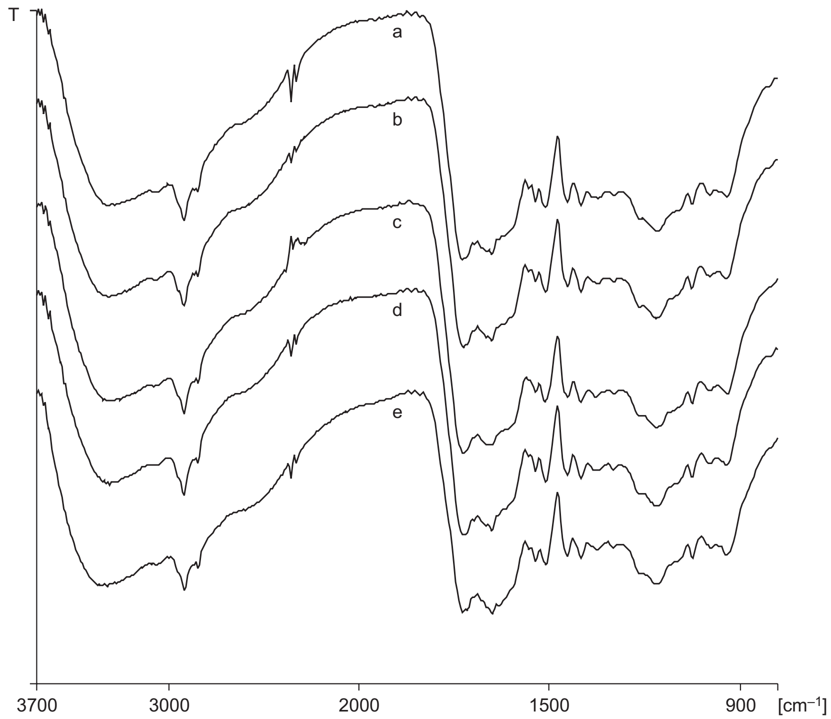

Fig. 2. FT-IR spectra of humic acids: a – no fertilisation; b – fertilisation with NPK; c – 10 Mg · ha^{-1} of dry matter of sludge; d – 20 Mg · ha^{-1} of dry matter of sludge; e – 30 Mg · ha^{-1} of dry matter of sludge

the aromatic ring. In the 1600–1660 cm^{-1} range there can also occur bands derived from the vibration of groups C=N, and C=C coupled with the carbonyl group. The band at around 1540 cm^{-1} is determined by the presence of amine groups (deformation vibration), the 1500–1520 cm^{-1} band is characteristic for aromatic compounds (C=C). The band in the range of wavenumbers1440–1460 cm^{-1} corresponds to the deformation vibration of groups $-CH_3$ and $=CH_2$, the band 1400–1420 cm^{-1} points to the presence of groups O–C–O in esters and C=O in carboxyl acids, and OH groups. The 1320–1380 cm^{-1} band is connected with the presence of groups COO and CH bonds in the aliphatic chain. In the 1200–1280 cm^{-1} range there occur bands of C–O groups of phenols, carboxyl acids, esters and ethers, and the bands in the range 1130 cm^{-1} and 1030–1080 cm^{-1} point to the presence of polysaccharides. They can be also related to the presence of groups OCH_3 which occur in lignins and OH in alcohols [10, 12].

The IR spectra of humic acids in all the variants under study was generally similar (Fig. 2). An intensive 1710–1730 cm^{-1} band is noteworthy, indicating a high proportion of carbonyl groups in humic acid molecules. The proportion of C of carboxyl (165–180 range) and carbonyl (180–200 range) groups, calculated from the ^{13}CNMR results for soil samples from the control plot, from the plot where NPK fertilisers were applied and from one where sludge was applied at 30 t/ha, ranged from 19.4 to 21.5 % (Table 7).

Table 7

Percentage of selected forms of carbon in humic acids molecules calculated on the basis of ^{13}C NMR spectra

Object	C_{al}	C_{ar}	C_{lig}	C–CO	$C_{al}/C_{ar} + C_{lig}$
Control	51.2	21.4	5.9	21.5	0.53
NPK	50.4	22.4	7.8	19.4	0.59
30 Mg · ha^{-1} of the sludge*	53.1	21.7	5.4	19.8	0.51

* – waste activated sludge.

The carbon content in aliphatic structures ranged from 50.4 (HAs in the plot fertilised with NPK) to 53.1 (HAs in the plot fertilised with 30 Mg · ha^{-1} of sludge). Humic acids isolated from samples of soil fertilised with NPK contained slightly more carbon in aromatic structures as compared with HAs of the control and the pot with the largest dose of sludge. This results in higher values of $C_{ar}/(C_{al} + C_{lig})$ for HAs from the plot fertilised with NPK.

Conclusions

1. After three years of cultivation of *Miscanthus sacchariflorus* in soil fertilised with sludge, the organic carbon content was found to increase in the plot fertilised with the largest dose of the sludge fertiliser (30 Mg · ha^{-1}), while the nitrogen content increased in all the plots (10, 20, 30 Mg · ha^{-1}) fertilised with the organic waste material.

2. Humic acids of the organic matter, isolated from the plots fertilised with sludge and mineral fertilisers, were found to have a higher degree of internal oxidation

compared with the control. The variance of the other quality parameters of those acids was not significant.

3. Fertilisation of soil with sludge did not significantly affect the properties of humic acids in the third year of cultivation of the test grass, which is important in regard to the balance of the soil fertilisation in *Miscanthus* cultivation.

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PARAMETRY MATERII ORGANICZNEJ GLEBY POD UPRAWĄ TRAWY *Miscanthus sacchariflorus*

[1] Katedra Chemii Środowiska
Uniwersytet Technologiczno-Przyrodniczy w Bydgoszczy
[2] Katedra Gleboznawstwa i Chemii Rolniczej
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach
[3] Zakład Gleboznawstwa, Instytut Geografii
Uniwersytet Mikołaja Kopernika w Toruniu

Abstrakt: Badano wybrane właściwości materii organicznej poziomu próchnicznego gleby nawożonej osadem ściekowym w dawce 10, 20 i 30 Mg · ha^{-1} oraz mineralnie NPK, w trzecim roku po zbiorze biomasy trawy *Miscanthus sacchariflorus*. Analizowano: C_{org}, N_t w glebie, a w wydzielonych kwasach huminowych – skład pierwiastkowy, stosunki atomowe, stopień utlenienia wewnętrznego, wartość współczynnika absorpcji, właściwości hydrofilowo-hydrofobowe, widma w zakresie podczerwieni (IR) oraz ^{13}C NMR i na ich podstawie określono formy węgla. Stwierdzono zwiększenie zawartości węgla związków organicznych na obiekcie nawożonym największą dawką osadu ściekowego (30 Mg · ha^{-1}), a azotu na wszystkich obiektach nawożonych tym odpadowym materiałem organicznym, w stosunku do obiektu kontrolnego. Kwasy huminowe materii organicznej wydzielone z gleby obiektów nawożonych osadem ściekowym i mineralnie NPK cechowały się wyższymi wartościami stopnia utlenienia wewnętrznego. Zróżnicowanie pozostałych parametrów jakościowych tych kwasów nie było jednoznaczne. Nawożenie osadem ściekowym nie wpłynęło istotnie na zmianę właściwości kwasów huminowych w trzecim roku uprawy trawy testowej, co jest ważne z punktu widzenia stanu równowagi nawożonej gleby.

Słowa kluczowe: *Miscanthus sacchariflorus*, osady ściekowe, materia organiczna, widma IR, HPLC, ^{13}C NMR

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Elżbieta MALINOWSKA[1] and Dorota KALEMBASA[1]

CONTENT OF SELECTED ELEMENTS IN *Miscanthus sacchariflorus* (Maxim.) Hack BIOMASS UNDER THE INFLUENCE OF SEWAGE SLUDGE FERTILIZATION

ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW W TRAWIE *Miscanthus sacchariflorus* (Maxim.) Hack POD WPŁYWEM NAWOŻENIA OSADEM ŚCIEKOWYM

Abstract: The field experiment consisted in the examination of the sewage sludge and mineral fertilization application on contents of some elements: Fe, Mn, Mo, Li, Ti, Ba and Sr in stalks and leaves of *Miscanthus sacchariflorus* grass cultivated in the first year as well as in the whole biomass harvested in the second year. Total elements concentrations were analyzed by means of ICP-AES technique after combustion in a muffle furnance at 450 °C. Much higher concentrations of analyzed elements in leaves of amur silvergrass than in its stalks was found. More molybdenum, and less iron and titanium was recorded in the second year than in the first year of the experiment. Contents of other elements determinated in biomass were at similar levels in both years of cultivation.

Uptake and accumulation of selected elements in yields of amur silvergrass was higher in the first as compared with the second experimental year. Leaves showed higher uptake of Fe, Mn, Li, and Ti due to sewage sludge fertilization, while stalks uptake more Mo and Sr.

Keywords: *Miscanthus sacchariflorus,* iron, manganese, molybdenum, lithium, titanium, barium, strontium, waste activated sludge

Introduction

Some of several tens of grass species of *Miscanthus* genus are among perennial plants cultivated for energetic purposes [1–5]. Grass of *Miscanthus* genus is one of the few ones with C_4 photosynthesis mechanisms, and under European conditions, it is very resistant towards majority of plant diseases and pests [4]. Due to its properties, it is characterized by high industrial and energetic values, which are comparable with those for firewood, therefore it is considered as a valuable source of renewable energy [6].

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[1] Department of Soil Science and Agricultural Chemistry, Siedlce University of Natural Science and Humanities, ul. B. Prusa 14, 08–110 Siedlce, Poland, phone: +48 25 643 12 87, email: kalembasa@uph.edu.pl

Searching for new ways to achieve energy carriers is more often focused on an agriculture [7–9]. These plants distinctly react towards fertilization, namely organic one, and among other in a form of sewage sludge. Own studies involving many amur silvergrass species as well as other authors [10–15] reveal that fertilization using sewage sludge had positive influence on yielding of *Miscanthus sacchariflorus*. To produce large biomass, the plant needs great amounts of nutrients that can be supplied in a form of fresh sewage sludge instead of mineral fertilization. Fertilizing with that organic material is one of more efficient methods for sewage sludge utilization, which ensures the safety for an environment and restoring nutrients to further turnover. Cultivation of amur silvergrass cultivation on a wide scale in Poland would make possible to reduce the amounts of stored sludge, although many authors warn against its strong expansion, particularly in field cultivation [16–18]. Higher costs for fossil fuels output and allocation of deposits – namely of crude oil and natural gas – has to lead to the stress within whole worldwide economics. At increasing deficiency of the firewood, growing the energetic plant species, including silvergrass (*Miscanthus*), may in part satisfy the expectations on energy markets. Cultivating the fast-growing plants makes a challenge for farmers by promising the higher income and maintaining the employment. According to data in European Directive 2003/30/EC, energy from biological sources would be 5.75 % of the total amount of energy from fossil fuels in 2010. Moreover, producing the biomass of energetic plant species contributes to greenhouse effect reduction by means of CO_2 binding [19] and sequestration of carbon compounds in a soil [20].

The study was aimed at evaluating the influence of different rates of sewage sludge on contents of selected elements in biomass of *Miscanthus sacchariflorus* grass in the first and second years of field cultivation.

Material and methods

The experiment was laid out in spring 2005 on light soil of loamy sand granulometric composition (according to PN-R-04033) that was characterized by the following properties: $pH_{KCl} = 6.60$, organic carbon content 30.5 $g \cdot kg^{-1}$, total content of nitrogen 1.85 $g \cdot kg^{-1}$, total contents of some selected elements [$mg \cdot kg^{-1}$ soil]: Fe – 5186; Mn – 146; Mo – 0.231; Li – 1.70; Ti – 49.42; Ba – 82.18; Sr – 29.06, total amounts of those elements were determined by means of ICP-AES technique after soil dry digestion in muffle furnace at 450 ^oC. Plots of 2 m^2 area in three replications were separated in completely randomized pattern. Amur silvergrass (*Miscanthus sacchariflorus*) was the tested plant.

The experiment included following fertilization objects:

– control (with no fertilization);

– mineral NPK (mineral nitrogen was applicated in a form of urea according to the amount of nitrogen contained in 20 $Mg \cdot ha^{-1}$ d.m. of sludge);

– waste activated sludge at 10 $Mg \cdot ha^{-1}$ d.m.;

– waste activated sludge at 20 $Mg \cdot ha^{-1}$ d.m.;

– waste activated sludge at 30 $Mg \cdot ha^{-1}$ d.m.

The experimental objects were fertilized with phosphorus (triple superphosphate) and potassium fertilizer (potassium sulfate) maintaining the ratio N : P : K as 1 : 0.8 : 1.2. The chemical composition of sewage sludge indicated its usefulness for plant nutrition heavy metals and trace elements contents were at the levels acceptable (Table 1). Chemical composition of the sewage sludge indicated its usefulness for plant nutrition Decree of Environment Ministry [21]. Analyzed the sludge contained a limit (far from the critical value) of heavy metals [21, 22]. This sediment was characterized by, among others relatively high content of zinc (1453 mg $\cdot$ kg^{-1}), dominant over the other heavy metals. Czekala and Jakubus [23] found significantly higher amounts of this metal in sewage sludge, often exceeding the allowable amount.

Table 1

The content of selected elements in waste activated sludge

pH_{KCl}	Dry matter [%]	N	P	K	Ca	Mg	S	Na	Pb	Cd	Cr
		[g $\cdot$ kg^{-1} d.m.]							[mg $\cdot$ kg^{-1} d.m.]		
6.5	25	50.2	26.1	3.50	39.5	7.62	5.67	0.697	98.7	2.70	20.5
[mg $\cdot$ kg^{-1} d.m.]											
Fe	Mn	Mo	B	Li	Ti	Ba	Sr	Zn	Ni	Cu	Co
8991	607	3.64	8.12	6.31	32.7	91.0	83.1	1453	52.7	101	4.49

Prior to rhizomes planting, the sludge was mixed with soil up to 25 cm depth. The biomass harvest was performed in December 2005 and 2006 after the first and second cultivation years. In the first year, chemical compositions of stalks and leaves were analyzed separately, while all biomass (stalks plus leaves) in the second year. Plant samples in a from of 10 leafy steams were collected after each silver grass harvest. Plant material was ground to the particle size of 0.25 mm and 1 g was weighed out into the stoneware crucible, then organic substance was dry digested at 450 °C in muffle furnace to raw ash in crucible to dissolved the ash and decomposed hydrochloric acid (1:1) was added carbonate. Such prepared basic solution was subjected to determination of total contents of Fe, Mn, Mo, Li, Ti, Ba, and Sr by means of ICP-AES technique.

Harvested yields of biomass and contents of selected elements served to calculate their uptakes. Stalks and leaves yields in the first amur silvergrass cultivation year were published in the paper [24]. Results were statistically processed; differences between mean values for plant parts as well as fertilization were verified using Fisher-Snedecor's test, and in the case of their significance, the $LSD_{0.05}$ values were calculated according to Tukey's test.

Results and discussion

System of weather conditions during plant's vegetation, namely that of C_4 pathway, is one of the most important factors that affect the rate of their development and yield size [25]. Mean air temperatures and rainfall sums in 2005–2006 in middle-eastern Poland (data for Siedlce) are presented in Table 2. Average monthly temperatures in April, May, June, July, and August were higher than long-term average, which had positive effects on silvergrass growth. Low rainfall sum in April (by 31.2 mm lower than long-term average level) caused that most plants started their growth at the end of April and beginning of May. Irregular rainfall distribution since July till October, almost twice as high in some years as long-term average, made the shoots were bent due to leaves weight and in consequence their fall.

Table 2

Temperature [^{o}C] and rainfall [mm] during vegetation period of *Miscanthus sacchariflorus*

Year	Month							
	April	May	June	July	August	September	October	Sum Mean
Temperature average in month [^{o}C]								
2005	8.60	13.0	15.9	20.2	17.5	15.0	8.51	16.5
2006	8.40	13.6	17.2	22.3	18.0	15.4	9.20	14.9
Mean	8.50	13.3	16.6	21.3	17.8	15.2	8.90	15.7
Mean for multiyears	7.70	10.0	16.1	19.3	18.0	13.0	7.50	
Rainfalls [mm]								
2005	12.3	64.7	44.1	86.5	45.4	15.8	0.0	269.0
2006	29.8	39.6	24.0	16.2	227.6	22.0	7.20	366.4
Mean	21.1	52.2	34.1	51.4	136.5	18.9	3.60	317.8
Mean for multiyears	52.3	50.0	68.2	45.7	66.8	60.7	68.3	

In the first of year experimental, much higher contents of analyzed selected elements were recorded in amur silvergrass leaves than stalks (Table 3).

Table 3

Total contents of selected elements [mg · kg^{-1} d.m.] in the biomass of *Miscanthus sacchariflorus* in the first year of the field experiment

Fertilization object	Yield [Mg · ha^{-1}]	Fe	Mn	Mo	Li	Ti	Ba	Sr
	Leaves							
Control object	1.12	293.2	17.93	1.06	1.47	3.44	7.67	23.27
NPK	1.57	276.2	16.41	0.880	1.41	3.54	8.91	27.61
10 Mg · ha^{-1}	1.56	334.8	15.48	1.12	2.50	4.86	8.45	23.16
20 Mg · ha^{-1}	1.60	322.4	19.13	0.924	1.85	3.95	8.55	25.81
30 Mg · ha^{-1}	1.53	360.4	22.92	0.846	2.27	4.67	8.83	26.53
Mean	1.48	317.4	18.37	0.966	1.90	4.09	8.48	25.28

Table 3 contd.

Fertilization object	Yield [$Mg \cdot ha^{-1}$]	Fe	Mn	Mo	Li	Ti	Ba	Sr
	Stalks							
Control object	0.690	61.72	6.07	0.093	2.16	0.537	2.36	8.44
NPK	1.10	60.08	5.28	0.090	3.51	0.491	2.14	7.47
10 $Mg \cdot ha^{-1}$	0.980	49.88	3.66	0.156	3.05	0.448	1.66	8.50
20 $Mg \cdot ha^{-1}$	1.15	55.63	7.14	0.271	2.50	0.429	2.48	10.11
30 $Mg \cdot ha^{-1}$	0.980	51.43	3.73	0.116	1.90	0.431	1.99	9.31
Mean	0.980	55.75	5.18	0.145	2.62	0.467	2.13	8.77
$LSD_{0.05}$ for:								
A – fertilization	0.018	9.18	0.575	0.056	0.564	0.117	0.309	1.03
B – plant's parts	0.008	20.83	1.30	0.126	n.s.	0.226	n.s.	2.35
A/B – interaction	0.026	29.46	1.84	0.178	n.s.	0.376	0.990	3.32
B/A – interaction	0.017	20.54	1.29	0.124	n.s.	0.262	0.690	2.31

n.s. – non significant difference; 10, 20, 30 $Mg \cdot ha^{-1}$ d.m. of waste activated sludge; yield of biomass published in the paper [14].

Mean concentrations in leaves can be lined up in following sequence: ($mg \cdot kg^{-1}$ d.m.): Fe (317.4) > Sr (25.28) > Mn (18.37) > Ba (8.48) > Ti (4.09) > Li (1.90) > Mo (0.966), while in stalks: Fe (55.75) > Sr (8.77) > Mn (5.18) > Li (2.62) > Ba (2.13) > Ti (0.467) > Mo (0.145).

Statistical analysis revealed significant influence of fertilization on contents of all tested elements in studied parts of amur silvergrass. More Fe, Mn, Mo, Ti, Ba, and Sr and much less Li was found in leaves than stalks. Sewage sludge fertilization differentiated contents of tested elements in leaves and stalks of amur silvergrass as compared with the control object. No univocal effect of sewage sludge rate on concentrations of selected microelements in studied parts of the grass was observed.

In the second cultivation year, analysis of chemical composition of the whole plant's biomass (leaves + stalks) harvested in December was made (Table 4). Mean contents of trace elements in question was lined up in the following sequence ($mg \cdot kg^{-1}$ d.m.): Fe (259.1) > Sr (20.36) > Mn (18.01) > Ba (8.55) > Li (2.47) > Ti (1.84) > Mo (1.64). It was found much more molybdenum, and less iron and titanium than in the first year of experiment; other elements were present at similar levels in both study terms. Fertilization considerably affected the iron and manganese contents in biomass of tested grass; in the case of other microelements, no significant influence of sludge fertilization was recorded. Kotecki [26] achieved similar iron content and highest manganese concentration in biomass of amur silvergrass. Own experiments revealed that sewage sludge fertilization did not cause the excessive accumulation of studied elements in analyzed grass [15]. Krzywy et al [27] obtained similar manganese content and significantly lower iron content in the biomass of *Miscanthus* fertilized with sewage sludge.

Table 4

Total contents of selected elements [mg · kg^{-1} d.m.] in the biomass of *Miscanthus sacchariflorus* in the second year of the field experiment

Fertilization object	Yield [Mg · ha^{-1}]	Fe	Mn	Mo	Li	Ti	Ba	Sr
Control object	1.65	291.6	13.36	2.12	3.03	2.01	8.06	22.10
NPK	1.95	238.1	17.52	1.53	1.58	1.60	7.59	19.64
10 Mg · ha^{-1}	1.67	230.6	13.09	1.50	3.11	1.64	7.89	19.84
20 Mg · ha^{-1}	1.71	241.7	18.29	1.56	2.31	2.01	9.21	20.42
30 Mg · ha^{-1}	1.58	293.6	27.76	1.50	2.33	1.95	10.02	19.80
Mean	1.71	259.1	18.01	1.64	2.47	1.84	8.55	20.36
LSD$_{0.05}$	0.999	60.96	4.65	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. – non significant difference.

In the first cultivation season, plants uptake of Fe, Li, Ti, Ba, and Sr with sum of the yield was higher than in the second year, whereas uptake of Mn was 9 times lower and that of molybdenum and boron slightly lower (Tables 5 and 6).

Table 5

Uptake of selected elements [g · ha^{-1}] with yields of *Miscanthus sacchariflorus* leaves and stalks in the first year of the field experiment

Fertilization object	Fe	Mn	Mo	Li	Ti	Ba	Sr
	Leaves						
Control object	328.3	20.08	1.19	1.65	3.85	8.59	26.06
NPK	433.6	25.76	1.38	2.21	5.56	13.99	43.35
10 Mg · ha^{-1}	522.3	24.15	1.75	3.90	7.57	13.18	36.13
20 Mg · ha^{-1}	515.8	30.61	1.48	2.96	6.32	13.68	41.30
30 Mg · ha^{-1}	551.4	35.07	1.29	3.47	7.15	13.51	40.59
Mean	470.3	27.13	1.42	2.84	6.09	12.59	37.49
Stalks							
Control object	42.59	4.19	0.064	1.49	0.371	1.63	5.82
NPK	66.09	5.81	0.099	3.86	0.540	2.35	8.22
10 Mg · ha^{-1}	48.88	3.59	0.153	2.99	0.439	1.63	8.33
20 Mg · ha^{-1}	63.98	8.21	0.312	2.88	0.493	2.85	11.63
30 Mg · ha^{-1}	50.40	3.66	0.114	1.86	0.422	1.95	9.12
Mean	45.87	5.09	0.148	2.62	0.453	2.08	8.62
Sum							
Control object	370.9	24.27	1.25	3.14	4.22	10.22	31.88
NPK	499.7	31.57	1.48	6.07	6.10	16.34	51.57
10 Mg · ha^{-1}	571.2	27.74	1.90	6.89	8.01	14.81	44.46
20 Mg · ha^{-1}	579.8	38.82	1.79	5.84	6.81	16.53	52.93
30 Mg · ha^{-1}	601.8	38.73	1.40	5.33	7.57	15.46	49.71
Mean	524.7	32.22	1.56	5.45	6.54	14.67	46.11

Table 6

Uptake of selected elements [g · ha^{-1}] with yields of *Miscanthus sacchariflorus* leaves and stalks in the second year of the field experiment

Fertilization object	Fe	Mn	Mo	Li	Ti	Ba	Sr
Control object	481.1	22.04	3.50	4.99	3.32	13.30	36.47
NPK	464.3	34.16	2.98	3.08	3.12	14.80	38.30
10 Mg · ha^{-1}	385.1	21.86	2.51	5.19	2.74	13.18	33.13
20 Mg · ha^{-1}	241.7	31.28	2.67	3.95	3.44	15.75	34.92
30 Mg · ha^{-1}	463.9	43.86	2.37	3.68	3.08	15.83	31.28
Mean	407.3	30.64	2.81	4.18	2.59	14.57	34.82

More Fe, Mn, Li, and Ti was recorded in amur silvergrass leaves due to sewage sludge fertilization, while more Mo and Sr in stalks. Woloszyk [28] says that the grass may accumulate significant amounts of elements, especially at high fertilization with sewage sludge. Download and use of elements by plants from sewage sludge is dependent on many factors: water content, soil temperature, to microbiological activity [28, 29]. Each of these factors can stimulate, or inhibit mineral uptake by plants and affect their chemical composition [30]. Uptake of studied trace elements with amur silvergrass biomass yield can be lined up in the following sequence: the first year: Fe > Sr > Mn > Ba > Ti > Li > Mo; the second year: Fe > Mn > Sr > Ba > Li > Mo > Ti.

Kalembasa and Malinowska [31] found that *Miscanthus* of rhizomes and roots contain much more heavy metals and other elements than the stalks and leaves. *Miscanthus sacchariflorus* is therefore particularly suitable for the cultivation of the plant using sewage sludge. Accumulation of elements namely toxic, in roots is very positive phenomenon at energetic utilization of plants.

Conclusions

1. Leaves of *Miscanthus sacchariflorus*, in the first year of experiment, contained much higher levels of Fe, Mn, Mo, Ti, Ba, and Sr than stalks. No univocal influence of sewage sludge rate on concentrations of selected elements in studied grass parts was observed.
2. Much more Mo, and less Fe and Ti were found in the biomass of tested plant in the second vs. the first cultivation year. Contents of other analyzed elements were similar in both years.
3. Uptake and accumulation of selected elements in yields of amur silvergrass was higher in the first as compared with the second experimental year. Leaves showed higher uptake of Fe, Mn, Li, and Ti due to sewage sludge fertilization, while stalks Mo and Sr.

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ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW W TRAWIE *Miscanthus sacchariflorus* (Maxim.) Hack POD WPŁYWEM NAWOŻENIA OSADEM ŚCIEKOWYM

Katedra Gleboznawstwa i Chemii Rolniczej
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach

Abstrakt: W doświadczeniu polowym badano wpływ nawożenia osadem ściekowym i nawozami mineralnymi (dla porównania) na zawartość wybranych pierwiastków: Fe, Mn, Mo, Li, Ti, Ba i Sr w łodygach i liściach trawy *Miscanthus sacchariflorus,* uprawianej w I roku oraz w całej biomasie, zebranej w II roku. Zawartość ogólną wymienionych pierwiastków oznaczono metodą ICP-AES, po mineralizacji na sucho w piecu muflowym, w temperaturze 450 °C. Stwierdzono kilkakrotnie większą zawartość analizowanych pierwiastków w liściach miskanta cukrowego niż w łodygach. W II roku zanotowano więcej molibdenu, mniej żelaza i tytanu niż w I roku eksperymentu. Zawartość pozostałych pierwiastków była na zbliżonym poziomie w obu terminach badań.

Pobranie i wyniesienie badanych pierwiastków z plonem miskanta cukrowego było większe w I roku uprawy, w stosunku do II roku. Liście pobierały więcej Fe, Mn, B, Li i Ti pod wpływem nawożenia osadem ściekowym, łodygi Mo i Sr.

Słowa kluczowe: *Miscanthus sacchariflorus*, żelazo, mangan, molibden, lit, tytan, bar, stront, osad ściekowy

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Barbara SAWICKA[1] and Stanisław KALEMBASA[2]

FLUCTUATION OF PROTEIN NITROGEN LEVEL IN TUBERS OF *Helianthus tuberosus* L. CAUSED BY VARYING LEVELS OF NITROGEN FERTILISATION

FLUKTUACJA AZOTU BIAŁKOWEGO W BULWACH *Helianthus tuberosus* L. W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO

Abstract: The effect of nitrogen fertilisation against potassium and phosphorus fertilisation on the total and true protein content in tubers of *Helianthus tuberosus* L. was examined. Two cultivars of the test plant (Albik and Rubik) were cultivated for 3 years in a field experiment on slightly acidic soil with a granulometric composition of loamy sand. The experimental objects were: 1) control – no fertilisation; 2) PK fertilisation at kg $\cdot$ ha^{-1}; 52.4 – P and 149.4 – K and (against PK fertilisation) four levels of nitrogen fertilisation: 50, 100, 150 and 200 kg $\cdot$ ha^{-1}. The total protein in tubers was calculated by multiplying the nitrogen content determined by the Kjeldahl method by 6.25, while the true protein content was determined by the Bernstein method. The protein content in tubers of the Jerusalem artichoke cultivars under study was significantly affected by their genetic features. The Rubik cultivar was found to contain significantly more of both forms of protein than the Albik cultivar. Irrespective of the cultivar, phosphorus and potassium fertilisation increased the total protein content compared with the control. The application of nitrogen at a dose as low as 50 kg $\cdot$ ha^{-1} increased the content of both forms of protein.

Keywords: Jerusalem artichoke, cultivar, total protein content, protein proper, share of proper protein in total protein

Introduction

Proteins are nutrients which determine the metabolism in live organisms. They are chemical compounds formed by the polycondensation of 18–20 amino acids. These are macromolecular biopolymers (with molecular weights ranging from approx. 10,000 to

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[1] Department of Plant Production Technology and Commodity, University of Life Sciences in Lublin, ul. Akademicka 15, 20–950 Lublin, Poland, email: barbara.sawicka@up.lublin.pl

[2] Department of Soil Science and Agricultural Chemistry, Siedlce University of Natural Sciences and Humanities, ul. B. Prusa 14, 08–110 Siedlce, Poland, email: kalembasa@uph.edu.pl

several million Daltons) and biological polycondensates, made up of amino acid residues connected by peptide bonds -CONH-. Proteins occur in all live organisms and in viruses. Protein synthesis is mediated by special cell organelles called ribosomes. The number of amino acid residues in a single polypeptide chain is larger than 100 and the entire molecule can contain many polypeptide chains [5, 8, 21]. Due to their multiple functions (enzymatic catalysis, transport, storage, buffers, construction material, immune functions, regulators, etc.) proteins are very important. Crop plants, including Jerusalem artichoke, are a good source of proteins. Large doses of nutrients in the form of fertilisers, mainly nitrogen, phosphorus and potassium, increase the productivity of overground parts and tubers of *Helianthus tuberosus* L. [17, 19]. However, such large doses of those nutrients are suspected of reducing protein content and to increase nitrate accumulation in plants. The aim of the study was to examine the effect of nitrogen fertilisation at different doses used against potassium and phosphorus fertilisation on the total and true protein content in tubers of *Helianthus tuberosus* L.

Materials and methods

A field experiment was conducted at the Field Experimental Station in Parczew in 2003–2005, on slightly acidic soil with a granulometric composition of loamy sand. The experiment was conducted by the randomised blocks method in triplicate and the following factors were analysed:

– primary factors – cultivars (2 cultivars: Albik and Rubik);

– secondary factors – nitrogen doses (5 nitrogen doses, kg · ha^{-1}), *ie* 0, 50, 100, 150 and 200 against a constant level of potassium and phosphorus fertilisation (52.4 kgP · ha^{-1} and 149.4 kgK · ha^{-1}). The mineral fertilisation was applied against manure fertilisation at 25 Mg · ha^{-1}.

Phosphorus was applied as a granulated superphosphate 19 % (P_2O_5), potassium – as potassium salt 60 % (K_2O), while nitrogen was applied as urea 46 %. The nitrogen

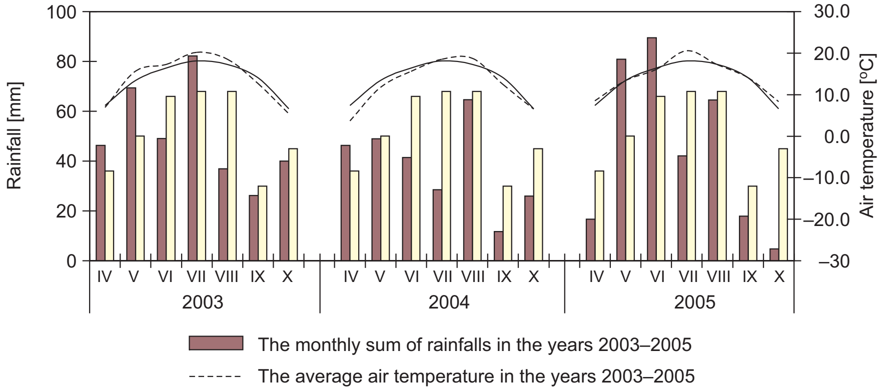

Fig. 1. Rainfalls and air temperature in vegetation periods of *Helianthus tuberosus* acc. to meteorological station of COBORU in Uhnin (years 2003–2005)

doses below and equal to 100 kg · ha^{-1} were applied on a one-off basis before tubers were planted; those above 100 kgN · ha^{-1} were applied twice: before tuber planting and after the plant sprouting, at phase 14 according to the BBCH scale. Tubers were planted in spring (on 26–27 April), spaced every 40 cm, with the rows spaced every 62.5 cm. The surface area of each plot was equal to 20 m^2. All the agricultural procedures were performed in accordance with the requirements for the crop. The weather conditions in the years of the experiment varied. The year 2003 was average in terms of the rainfall level, while 2004 was dryish (Fig. 1) and 2005 was extremely dry. 50 tubers of different sizes were taken for laboratory analyses during the autumn (mid-October) harvest. The following were determined in the fresh matter of tubers: dry matter by drying, total protein – by converting the nitrogen content (× 6.25) determined by the Kjeldahl method and true protein – by the Bernstein method [1]. The significance of the sources of variance was tested with the Fisher-Snedecor test and the significance of differences between average values was analysed by Tukey's test.

Experiment results

The genetic features of the cultivars under study proved to significantly affect the total and true protein in tubers of *Helianthus tuberosus*. The Rubik cultivar accumulated significantly more total and true protein in its tubers than the Albik cultivar. The feature variance was low, with the total protein content being more stable in the Rubik cultivar, while the true protein content was more stable in the Albik cultivar (Table 1).

Table 1

Effect of cultivars on the content of total and proper protein in the tubers of *Helianthus tuberosus* [g · kg^{-1} d.m.]

Specification	Cultivar				
	Albik		Rubik		$LSD_{0.05}$
	mean	C_V*	mean	C_V	
Total protein	111.36	10.44	121.72	8.18	3.84
Proper protein	58.38	7.66	63.23	11.04	2.06

* Variability coefficient; d.m. = dry matter.

Mineral fertilisation significantly modified the average total protein content and true protein content in both cultivars of *Helianthus tuberosus* tubers (Table 2, Fig. 2).

Phosphorus and potassium fertilisation increased the content of total protein in tubers as compared with the control (no fertilisation), while the content of true protein did not change significantly (Table 2). Nitrogen fertilisation had a decisive effect on protein content. A significant increase in the total and true protein content was observed in tubers harvested on plots fertilised with 50 kgN · ha^{-1}, against constant phosphorus and potassium fertilisation. Larger doses of nitrogen did not cause the content of both protein forms to increase so clearly. The regression analysis showed the existence of

Table 2

Effect of mineral fertilisation and years on the content of total and proper protein in the tubers of *Helianthus tuberosus* [g · kg^{-1} d.m.]

Fertilisation [kg · ha^{-1}]	Total protein				Proper protein			
	Years			Mean	Years			Mean
	2003	2004	2005		2003	2004	2005	
0	85.50	110.40	90.48	95.46	42.38	64.92	53.65	53.65
$P_{44}K_{125}$	95.75	129.78	86.74	104.09	41.20	68.07	54.63	54.63
$N_{50}P_{44}K_{125}$	120.23	159.27	104.02	127.84	50.43	76.08	63.26	63.26
$N_{100}P_{44}K_{125}$	116.18	137.30	87.37	113.61	51.27	71.92	61.59	61.59
N_{150} $P_{44}K_{125}$	136.23	161.60	106.19	134.68	57.97	67.98	62.98	62.98
N_{200} $P_{44}K_{125}$	132.95	139.40	98.35	123.57	73.27	64.17	68.72	68.72
$LSD_{0.05}$	34.56			11.52	18.52			6.17
Mean	114.47	139.63	95.53	116.54	52.75	68.86	60.80	60.80
$LSD_{0.05}$	5.76				3.09			

a third order curvilinear relationship between the content of total and true protein and mineral fertilisation, with R^2 equal to: 0.87 and 0.74, respectively (Fig. 2).

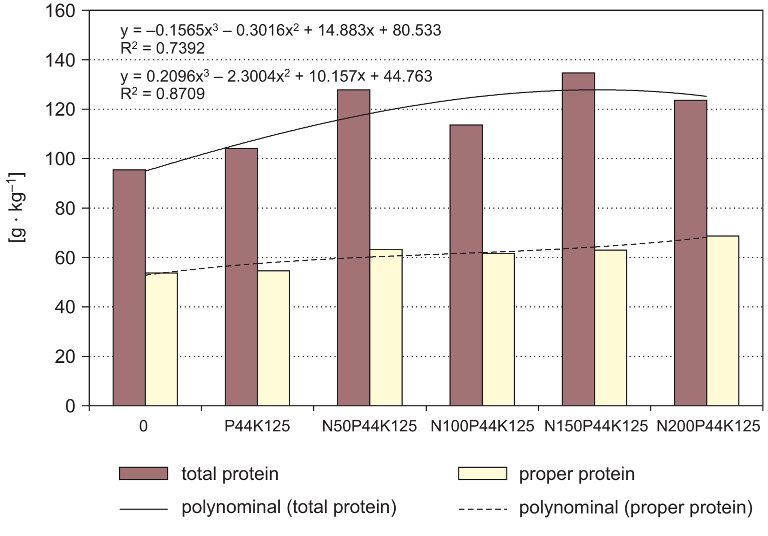

Fig. 2. Effect of mineral fertilization on total protein and proper content in tubers of *Helianthus tuberosus* (Mean 2003–2005)

The plant cultivars under study reacted differently to mineral fertilisation in regard to the accumulation of both protein forms. The phosphorus and potassium fertilisation did not affect the total and true protein content as compared with the control, whereas nitrogen fertilisation did. The total protein content in tubers of the Albik cultivar was found to increase on the plot fertilised with 150 kgN · ha^{-1}, while that in tubers of the

Rubik cultivar – on the plot fertilised with 50 kgN · ha^{-1} (Fig. 3). As compared with the control plot, the true protein content in tubers of the Albik cultivar was found to increase significantly on the plot fertilised with 50 kgN · ha^{-1}, while that in tubers of the Rubik cultivar increased significantly on the plot fertilised with 200 kgN · ha^{-1} (Fig. 4).

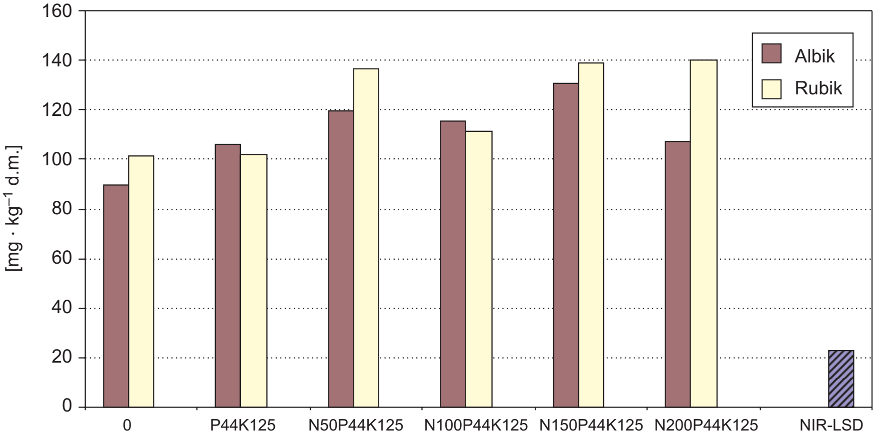

Fig. 3. Effect of cultivars and mineral fertilization on the content of total protein in the tubers of *Helianthus tuberosus* (mean 2003–2005)

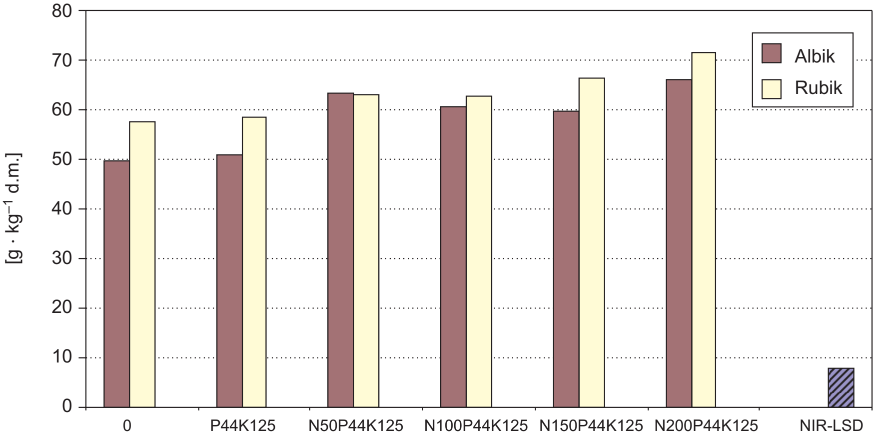

Fig. 4. Effect of variety and mineral fertilization on the content of protein proper in the tubers of *Helianthus tuberosus* (mean 2003–2005)

The effect of mineral fertilisation, compared with the control, depended on the weather conditions during the years of the study (Table 2). In the years 2003–2004, the total protein content increase was found to be caused by fertilisation with 50 kg of N · ha^{-1}, against phosphorus and potassium fertilisation. In 2005, with a drought lasting from July to the end of October (during the period of tuberisation and tuber growth), no significant changes in the total protein content were found to be caused by mineral fertilisation (Table 2). True protein content on plots fertilised with 200 kgN · ha^{-1} was found to increase only in 2003, when the air temperature was higher than the multi-year average and the total rainfall was close to the multi-year average. Phosphorus and potassium fertilisation alone did not significantly affect the value of the feature (Table 2).

The cultivar effect was observed together with the year of study (Table 3). The Rubik cultivar accumulated significantly more total protein in tubers than the Albik cultivar in 2003–2004, whereas no cultivar-related differences were observed in 2005. No year-related differences for the cultivars were recorded in the true protein content.

Table 3

Effect of cultivars and years on the content of selected elements in dry mass of aboveground parts of Jerusalem artichoke [g · kg^{-1} d.m.] (mean for 2003–2005)

Years	Total protein		Proper protein	
	Cultivars			
	Albik	Rubik	Albik	Rubik
2003	108.60	120.30	50.22	55.29
2004	133.00	146.20	66.54	71.27
2005	92.40	98.60	58.38	63.23
$LSD_{0.05}$	11.52		n*	

* not significant at $\alpha \leq 0.05$.

The proportion of true protein in the total protein, as an important factor in the evaluation of the protein value, depended on mineral fertilisation, cultivar and year of study, and was co-affected by those factors. Mineral fertilisation modified the value of the feature significantly. The proportion of true protein in total protein was found to decrease significantly on plots fertilised with 150 kgN · ha^{-1} as compared to the control. An analysis of regression showed the existence of the 5th order polynomial relationship between the proportion of true protein in total protein and fertilisation, with the determination coefficient $R^2 = 1.0$ (Fig. 5).

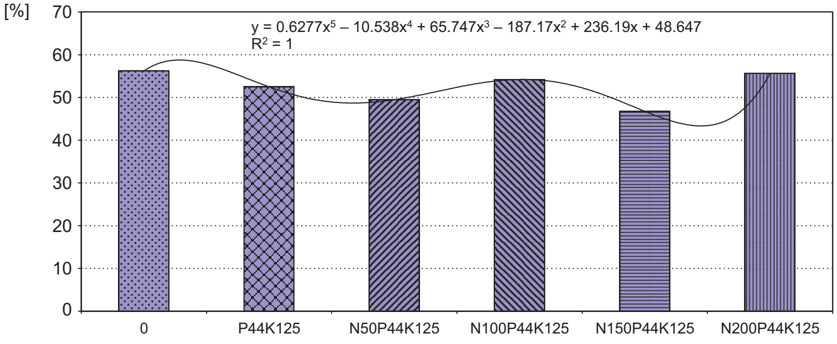

Fig. 5. Effect of mineral fertilization on the share of proper protein in total protein

The value of the feature also depended on the cultivar reaction on mineral fertilisation (Table 3). The highest proportion of true protein in total protein for the Rubik cultivar was found in the control plot; for the Albik cultivar it was on plots fertilised with 200 kg of N · ha^{-1}. The relationship for the Albik cultivar can be described by the 2nd order parabolic regression equation; for the Rubik cultivar – by the 4th order polynomial regression equation. The determination coefficients for those equations were equal to: R^2 = 0.81 and 0.99, respectively (Fig. 6). The highest proportion of true protein in total protein in 2003, with sufficient rainfall level, was caused by fertilisation at 200 kgN · ha^{-1}; in dry 2005 – by fertilisation with 100 kgN · ha^{-1}, and in extremely dry 2004 – on the control plot (Fig. 7).

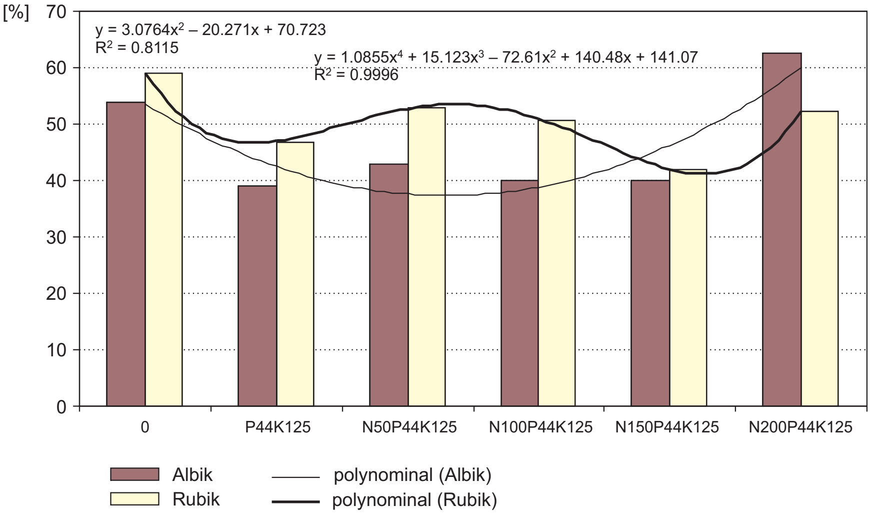

Fig. 6. Effect of mineral fertilization and variety on the proportion of proper protein to total protein

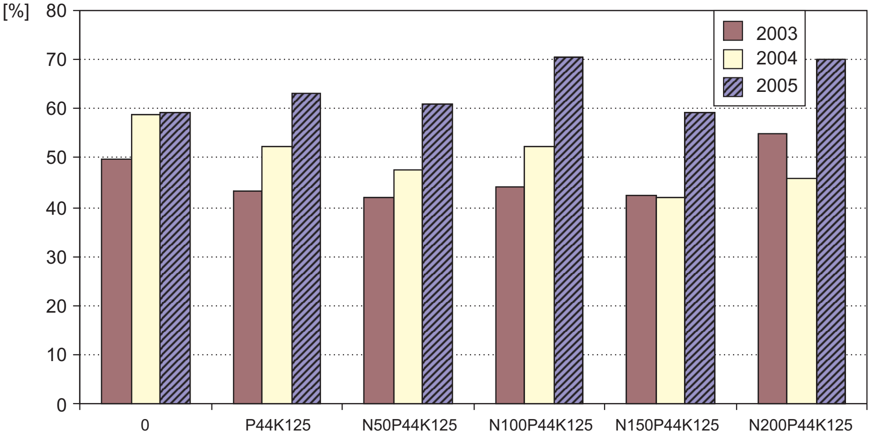

Fig. 7. Effect of mineral fertilization and years on the share of proper protein in total protein

Discussion

Proteins of tubers of *Helianthus tuberosus* stand out among plant proteins in terms of their biological value. They contain exogenous amino acids, including methionine and tryptophan [5]. The average content of total protein in dry matter of tubers of *Helianthus tuberosus* ranged from 78.38 to 170.80 $g \cdot kg^{-1}$, and that of true protein from 42.20 to 72.90 $g \cdot kg^{-1}$. According to Mikos-Bielak et al [13], the content of total protein in dry matter ranged from 87.1 to 134.3 $g \cdot kg^{-1}$, and that of true protein from 43.2 to 57.0 $g \cdot kg^{-1}$. The findings of a study by Florkiewicz et al [7] showed tubers of the species to contain 65–86 $g \cdot kg^{-1}$ of total protein. Those values are higher than the tabular data on the potato and root vegetables [5, 6]. A wider range of variance for total and true protein content in tubers is a result of the effect of biotic and abiotic factors, such as: cultivar-related factors, habitat conditions, agrotechnical factors and harvest time [3, 8, 9, 11].

Nitrogen fertilisation had a significant effect on total and true protein content in tubers of *Helianthus tuberosus*. The dose of 50 kg of $N \cdot ha^{-1}$ has proven to be a safe one, which does not reduce the protein content. These findings have been corroborated by a study conducted by Berghofer and Reiter [2]. An earlier study conducted by Sawicka [17, 19] showed that 100 $kgN \cdot ha^{-1}$ is such a dose in regard to tuber quality. In the opinion of Mikos-Bielak et al [13], nitrogen fertilisation increases the protein content in a plant and, in consequence, changes its amino acid profile. Changes of proportions between exogenous amino acids, especially lysine and methionine, seem to be of special importance as their deficit, according to many authors [5, 14–16], decreases the nutritional value of the proteins of *Helianthus tuberosus*.

The proportion of true protein in total protein in dry matter of *Helianthus tuberosus* tubers was equal to 46.8–56.2 % – depending on mineral fertilisation, 46.4–50.7 % – depending on the cultivar and 46.1–63.8 % – depending on the year of the experiment. True protein accounts for approx. 60 % of all the nitrogen-containing substances found in tubers of *Helianthus tuberosus*, with amides, peptones, ammonia, nitrates, nitrites accounting for the other 40 % [5, 13, 14, 20]. According to Praznik et al [15] and Florkiewicz et al [7], tubers harvested in autumn contain more protein than those harvested in spring. A different tendency was shown in a study conducted by Velverde et al [21] and Berghofer and Reiter [2], which probably resulted from differences in vegetation and cultivation conditions of various cultivars.

The cultivar-related properties modified the content of total and true protein. The results are similar to the findings of other studies [3, 4, 13, 17–19]. According to Velverde et al [21], the cultivar with reddish pigmentation of the peel contain more proteins that those with white peel. According to Sawicka [18, 19], the differences in protein content are caused by the phenotypic variation of the cultivars of Jerusalem artichoke, which is the combined effect of genetic and environmental variation. Jasiewicz et al [10] suggest that stressful conditions (prolonged drought or excessive rainfall) could disrupt chloroplast membrane function, which directly reduces productivity of the PS II system, which is the most sensitive indicator of the action of different stressful factors. The diversity of the conditions in which the species grows modifies internal regulation processes within the entire plant and its shoots.

The soil and weather conditions caused the greatest modifications of protein accumulation in tubers. Prosba-Bialczyk [16] found tubers of plants cultivated on heavy soil to contain less protein than those cultivated on medium-heavy soil. The variance of weather-related factors in the habitat (rainfall and temperature) can affect the tuber yield by modifying the rate of plant growth and development and can also affect their chemical composition [3, 18, 19, 21–23].

Conclusions

1. The cultivar-related properties of Jerusalem artichoke proved to significantly affect the total and true protein in tubers of the plant. The Rubik cultivar accumulated significantly more total and true protein than did the Albik cultivar. Elucidating the phenotypic variance of the cultivars of *Helianthus tuberosus* and identifying, separately, genetic and environmental variance, would enable choosing a cultivar with the highest stability of the desired feature for cultivation.
2. Irrespective of the cultivar, phosphorus and potassium fertilisation increased the total protein content in tubers of *Helianthus tuberosus* as compared with the control. The true protein content was not significantly changed by phosphorus and potassium fertilisation alone.
3. Total and true protein accumulation was changed differently in both cultivars by increasing doses of nitrogen. The total protein content in tubers of the Rubik cultivar was found to increase significantly on the plot fertilised with 50 $\text{kgN} \cdot \text{ha}^{-1}$, while that in tubers of the Albik cultivar increased on the plot fertilised with 150 $\text{kgN} \cdot \text{ha}^{-1}$. The true protein content in tubers of the Rubik cultivar was found to increase on the plot fertilised with 200 $\text{kgN} \cdot \text{ha}^{-1}$, while that in tubers of the Albik cultivar increased on the plot fertilised with 50 $\text{kgN} \cdot \text{ha}^{-1}$.
4. The dose of 50 $\text{kgN} \cdot \text{ha}^{-1}$ proved to be an environmentally safe dose of nitrogen fertilisation of Jerusalem artichoke. The proportion of true protein in total protein was found to decrease significantly on plots fertilised with 150 $\text{kgN} \cdot \text{ha}^{-1}$ against phosphorus and potassium fertilisation.

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FLUKTUACJA AZOTU BIAŁKOWEGO W BULWACH *Helianthus tuberosus* L. W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO

[1] Katedra Technologii Produkcji Roślinnej i Towaroznawstwa
Uniwersytet Przyrodniczy w Lublinie
[2] Katedra Gleboznawstwa i Chemii Rolniczej
Uniwersytet Przyrodniczo-Humanistyczny w Siedlcach

Abstrakt: Badano wpływ nawożenia azotem, na tle nawożenia fosforowo-potasowego, na zawartość białka ogólnego i właściwego w bulwach *Helianthus tuberosus*. W 3-letnim doświadczeniu polowym, na glebie lekko kwaśnej, o składzie granulometrycznym piasku gliniastego mocnego, oceniano dwie odmiany słonecznika bulwiastego (Albik i Rubik). Obiektami doświadczenia były: 1) kontrola bez nawożenia, 2) cztery poziomy nawożenia azotem: 50, 100, 150 i 200 kg $\cdot$ ha^{-1} na tle stałego nawożenia fosforowo-potasowego (52,4 kgP $\cdot$ ha^{-1} i 149,4 kgK $\cdot$ ha^{-1}) i pełnej dawki obornika (25 Mg $\cdot$ ha^{-1}). Całkowitą zawartość białka w bulwach określano, mnożąc zawartość azotu ogólnego oznaczonego metodą Kjeldahla przez 6,25, a zawartość białka właściwego oceniano metodą Bernsteina. Zawartość białka w bulwach *Helianthus tuberosus* zależała istotnie od cech genetycznych badanych odmian. Stwierdzono, iż odmiana Rubik zawierała znacznie więcej obu form białka niż odmiana Albik. Nawożenie fosforowo-potasowe zwiększało poziom białka ogólnego, w stosunku do obiektu kontrolnego, w bulwach *Helianthus tuberosus,* niezależnie od odmiany. Stosowanie azotu w dawce 50 kgN $\cdot$ ha^{-1} powodowało istotny wzrost zawartości obu form białka.

Słowa kluczowe: słonecznik bulwiasty, odmiany, zawartość białka ogólnego i właściwego, udział białka właściwego w białku ogółem

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Magdalena BANACH-SZOTT[1], Bożena DĘBSKA
and Grzegorz MROZIŃSKI

DECOMPOSITION RATE OF ANTHRACENE, FLUORENE, PYRENE AND CHRYSENE IN *LUVISOLS*

SZYBKOŚĆ ROZKŁADU ANTRACENU, FLUORENU, PIRENU I CHRYZENU W GLEBACH PŁOWYCH

Abstract: The aim of the paper was to determine the stability and intensity of decomposition of selected polycyclic aromatic hydrocarbons (PAHs) (anthracene, fluorene, pyrene, chrysene) in *Luvisols*. The study was carried out based on soil samples representative of the Kujawsko-Pomorskie Region, collected from areas exposed to and protected from direct contamination with PAHs. Soil samples were polluted with selected PAHs at the amount corresponding to 10 mg PAHs/kg. The PAHs-polluted soil samples were incubated for 10, 30, 60, 120, 180 and 360 days at the temperature of 20–25 °C and fixed moisture – 50 % of field water capacity. In this work High Performance Liquid Chromatography (HPLC) was applied. It was found that majority of PAHs decomposed within first 30 days of the experiment. Decomposition of fluorene and anthracene was much faster than for pyrene and chrysene. The lowest rate of PAHs decomposition was noted for the samples of the soil with the highest content of organic carbon, carbon of the fractions of humic acids and humins, which clearly points to an essential role of organic matter in the PAHs sorption processes.

Keywords: PAHs, HPLC, *Luvisols*

Introduction

Soil is an element of the natural environment in which there accumulates most hydrophobic organic pollutants, including polycyclic aromatic hydrocarbons (PAHs) [1]. PAHs are organic compounds which include from two to a dozen or so aromatic rings. The structures of respective PAHs differ in the pattern of benzene rings in the molecule and all the connected rings have two common carbon atoms. Ring bonds can occur in various spatial patterns: linear (*eg* anthracene, tetracene), cluster (*eg* pyrene, perylene) and angular pattern (phenanthrene, tetraphen) [2]. In general, PAHs, due to their specific physicochemical properties and the resultant low susceptibility to degradation are considered to be the so-called stable organic pollutants [3, 4].

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[1] Department of Environmental Chemistry, University of Technology and Life Sciences, ul. Bernardyńska 6, 85–029 Bydgoszcz, Poland, phone: +48 52 374 95 11, fax: +48 52 374 95 05, email: magdybe@poczta.fm, debska@utp.edu.pl

PAHs which occur in the soil environment can be of autogenic origin (formed as a result of humification) and exogenous, connected with the deposition of particulate matter from burning fossil fuels and biomass [5]. With that in mind, their presence ranges a lot, depending on the location, the type and method of soil use. The highest PAHs level (20000 μg/kg) is reported in the soils of big cities, along the roads, in the vicinity of industrial plants [6–9], while in the soils of arable fields, meadows and other agricultural land it usually does not exceed 100–400 μg/kg [3, 4, 10, 11].

According to Bojakowska and Sokolowska [12], the composition and amount of PAHs in soil is a derivative of two parallel processes which occurs in soil. The first one is the formation of new compounds as a result of the deposition of PAHs from the anthropogenic sources and the degradation of organic remains, while the second process covers a microbiological degradation of hydrocarbons which occurs with the participation of fungi, bacteria and Actinobacteria [13–15]. Interestingly, microbiological processes are the only way of a complete PAHs decomposition in the soil environment. PAHs in soils also undergo abiotic transformations, namely sorption, leaching, reactions with other compounds and photodegradation [6, 10, 16–19].

One of the key parameters affecting the amount of PAHs in soil is the content of organic matter. Organic matter shows a high sorption potential, and the stability of hydrocarbons in soil depends considerably on that process. As a result of sorption their mobility and PAHs availability to microorganisms decrease [20–22]. For the processes of PAHs sorption by organic matter not only its total content but also its quality composition are very important [23–26]. In general it is assumed that humins show a much greater sorption capacity towards PAHs than humic acids which, in turn, can sorb those hydrocarbons stronger than fulvic acids [26, 27].

Discussing PAHs transformations in soil, one shall note such factors as moisture, pH, temperature, oxygen access. PAHs demonstrate the highest stability in the air-dry soils (1 % field water capacity). The decomposition of PAHs is most intensive in the soils of the moisture falling within the range of 50 to 65 % field water capacity. In the soils of a higher water content (moisture above 65 % field water capacity) there is observed another decrease in the PAHs decomposition rate [28–30].

Yet another factor affecting the hydrocarbons decomposition intensity is temperature. The temperature optimal for bacteria and fungi decomposing PAHs is 1538 ^{o}C. Below the thermal optimum there occurs an inhibition of growth and soil microflora activity and a decrease in the biochemical reactions rate leading to the decomposition of PAHs [30–32].

The soil reaction, in turn, affects both the PAHs sorption processes and its biological activity. In the acid soils there is observed an increase in the intensity of the sorption process, and thus, a slow-down in the biological transformations of PAHs [23, 33–35]. The pH optimal for PAHs-decomposing microorganisms is 6.5–7.5. Although fungi develop well in the environment showing a slightly acid reaction (pH 4.5–6.0), the key role in the PAHs decomposition is played by bacteria. For that reason in the soils of low pH there occurs a decrease in the decomposition rate of those compounds, caused by a decreased activity of microorganisms.

The PAHs stability in soil also depends on the structure and the properties of hydrocarbons only. It was found that the higher the number of rings in the compound, and thus the higher the molecular weight, the lower the decomposition rate, which is related to an increased PAHs hydrophobicity and a strong adsorption by soil as well as the fact that they are more resistant to the microbiological attack [27, 36, 37].

The aim of the paper was to determine the stability and intensity of decomposition of selected polycyclic aromatic hydrocarbons (PAHs) (anthracene, fluorene, pyrene, chrysene) in *Luvisols*.

Materials and methods

Research material

The research was performed based on the *Luvisols* representative for the Region of the Kujawy and Pomorze, taken from the areas exposed to a threat and not exposed to any such threat of direct pollution with PAHs. The following soils were considered: samples number 3, 4 – sampled from the location of Bielawy (the soil exposed to a threat (3) and not exposed to any such threat (4) of direct effect of PAHs); sample number 5 – Orlinek (soil not exposed to the threat of PAHs pollution); sample number 7 – Slesin in the vicinity of Bydgoszcz (soil exposed to the effect of PAHs). The basic properties of soils used in the experiment are given in Table 1.

Table 1

The granulometric composition of the soils, the content of total organic carbon (TOC) and nitrogen (N_t) as well as the content of respective fractions of organic carbon

Sample	Percentage share of respective fractions			TOC	N_t	C_{HAs}	C_{FAs}	C_{HUMIN}
	2–0.05 mm	0.05–0.002 mm	> 0.002 mm	[g/kg]				
Luvisol (Lu3W)	82	18	0	12.80	0.66	3.906	2.094	5.546
Luvisol (Lu4W)	84	11	5	6.63	0.50	2.543	1.657	1.780
Luvisol (Lu5W)	75	19	6	11.74	1.03	3.424	2.906	4.388
Luvisol (Lu7W)	64	26	10	13.77	1.26	4.835	2.645	5.482

Soil samples were polluted with selected PAHs (fluorene, anthracene, pyrene and chrysene), at the amount corresponding to 10 mg PAHs/kg. The PAHs-polluted soil samples were incubated for 10, 30, 60, 120, 180 and 360 days at the temperature of 20–25 ^{o}C and fixed moisture (50 % of field water capacity).

After the completion of incubation, the samples were dried at room temperature were mixed and sieved.

Determining the PAHs content in soil

The content of PAHs (fluorene, anthracene, pyrene and chrysene) was determined in the samples of initial soils and after 10, 30, 60, 120, 180 and 360 days with the use of High Performance Liquid Chromatography. PAHs from the soils were extracted with cyclohexane applying the Soxhlet apparatus. The extracts were evaporated to dryness, and the rest was solved in 4 cm^3 acetonitrile (ACN).

The chromatographic separation of PAHs-containing solutions was made using the liquid chromatograph HPLC Series 200 provided by Perkin-Elmer equipped with a DAD (absorption) and FL (fluorescence) detector. There was applied an analytic column to separate PAHs provided by Waters of the particle size of 5 μm and 250 · 4.6 mm in size. The mobile phase was composed of eluent A: H_2O and eluent B: ACN. There was used a gradient separation program of a varied flow rate. The initial composition of the mobile phase was 70 % of eluent B and its concentration was growing linearly in the analysis time. Gradient was completed after 34 min when the content of eluent B was 100 %.

The extracts of the unpolluted (initial) soil samples were analysed with the fluorescence detector applying excitation wavelength λ_{ex} = 250 nm and emissions λ_{em} = 405 nm. The injection was 10 mm^3.

The extracts of the samples of soils polluted with PAHs, incubated 10, 30, 60, 120, 180 and 360 days were analysed with the absorption detector. The detection was made at λ = 254 nm. The injection was 100 mm^3.

The qualitative analysis of respective hydrocarbons was made with a comparison of soil extracts chromatograms with the model mixtures chromatograms.

The quantitative assays of fluorene, anthracene, pyrene and chrysene were made based on their model curves.

Results

The basic properties of the soil sampled from the sites exposed to and non-exposed to a direct effect of PAHs are given in Table 1. The highest content of total organic carbon (TOC), total nitrogen (Nt) and the share of fine fraction was found for *Luvisol* (Lu7W). The lowest content of TOC and Nt was reported for the soil sampled at Bielawy – Lu3W.

The contents of PAHs recorded for the initial samples of soils from the sites located close to transport routes (Lu3W and Lu7W) and away from them (Lu4W and Lu5W) are given in Table 2.

Table 2

Contents of PAHs in the soil samples

Sample	Fluorene	Anthracene	Pyrene	Chrysene	Sum
	[μg/kg]				
Lu3W	57.1	0.94	7.39	24.1	89.5
Lu3W’	2557	2501	2507	2524	10090
Lu4W	4.00	1.08	6.45	9.80	21.3
Lu4W’	2504	2501	2506	2510	10021
Lu5W	15.1	2.15	9.70	7.14	34.1
Lu5W’	2515	2502	2510	2507	10034
Lu7W	193	15.7	246	99.0	553
Lu7W’	2693	2516	2746	2599	10553

It was shown that the highest contents of the PAHs analyzed (anthracene, pyrene, chrysene and fluorene) were recorded for soil sample Lu7W, exposed to a direct effect of PAHs, while the soil sampled from the sites away from transport routes (Lu4W, Lu5W) showed the lowest PAHs contents, except for anthracene the lowest content of which was recorded for sample Lu3W.

Changes in the content of selected PAHs during incubation

The changes in the PAHs content reported for the *Luvisol* sampled at Bielawy in the vicinity of the transport route (Lu3) are given in Fig. 1 (sample Lu3W' refers to soil sample Lu3W additionally polluted with 4 selected PAHs (fluorene, anthracene, pyrene and chrysene), however, the amount of each of the PAHs was 2500 μg/kg). It was demonstrated that the contents of fluorene, anthracene, pyrene, chrysene in the samples decreased with the incubation time (Fig. 1). The highest decrease in PAHs, as compared with the initial content, was recorded for the sample after 10 days of incubation for which there was recorded a 64.6 % decrease in the initial content of fluorene, a 89.7 % decrease in the content of anthracene and by 59 % – pyrene. Over the 1st 10 days of incubation the lowest decrease was noted for chrysene – 28.7 % of its initial content. Over the successive periods of incubation: 10–30 and 30–60 days, the amount of chrysene which underwent decomposition accounted for 25.8 % and 31.2 % of its initial content, respectively. The lowest PAHs contents were recorded for the soil samples after 360 days of the experiment. The content of fluorene accounted for 3.8 % of its initial content, anthracene – 1.2 %, pyrene – 8.3 % and chrysene 9 %.

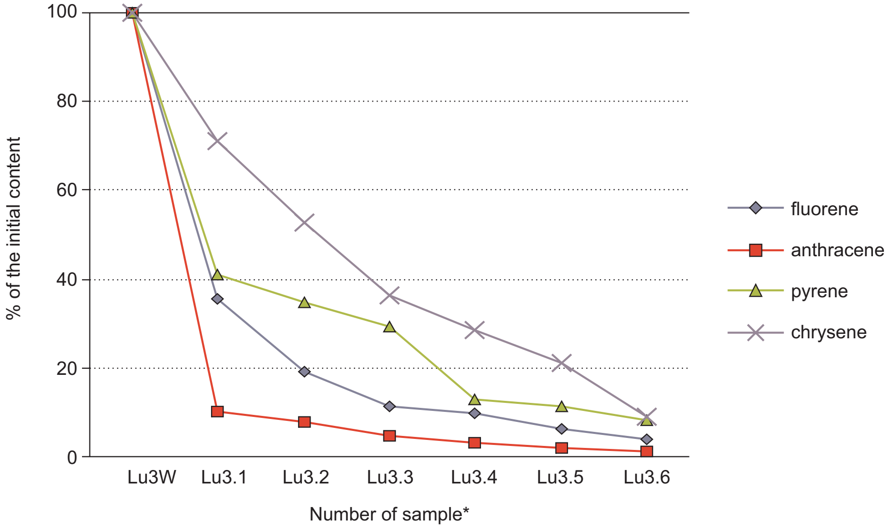

Fig. 1. Changes in the content of selected PAHs during incubation in the soil sampled at Bielawy (* – Lu3W – initial soil, Lu3.1 – after 10 days of incubation, Lu3.2 – 30 days, Lu3.3 – 60 days, Lu3.4 – 120 days, Lu3.5 – 180 days, Lu3.6 – 360 days

In the *Luvisol* sampled also at Bielawy (Lu4) (Fig. 2), however, at the sampling site away from the transport route, the amount of the decomposed PAHs over the first 10 days of incubation was higher than in soil samples Lu3 (Fig. 1). At the first stage of incubation in soil samples Lu4 91.6 % anthracene, 88.5 % fluorene, 72.7 % pyrene and 48.3 % chrysene was decomposed, as compared with their initial contents. The lowest PAHs contents were reported for the samples of soils after 360 days of the experiment: as for fluorene – 3.1 % of the initial content, anthracene – for 1.1 %, pyrene –for 6.3 %, and chrysene – for 7.1 %.

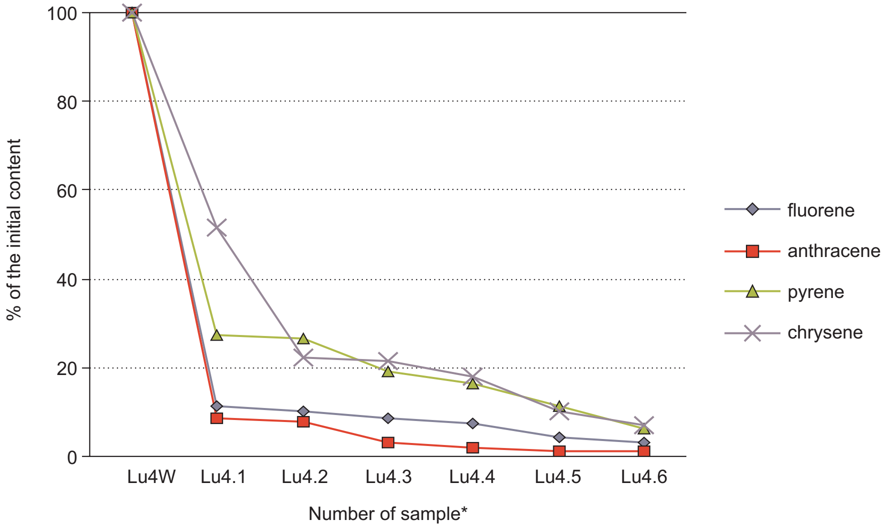

Fig. 2. Changes in the content of selected PAHs during incubation in the soil sampled at Bielawy (* – denotation as in Fig. 1)

The contents of fluorene, anthracene, pyrene, chrysene during the incubation in *Luvisol* sampled at Orlinek are given in Fig. 3. After the first 10 days of incubation the highest decrease was recorded for fluorene – 82.7 % of its initial content and anthracene – 85.1 %. The decrease in the content of pyrene and chrysene was much lower and it accounted for 56.7 % for pyrene and 41.8 % for chrysene. There was noted, however, a high decrease in those PAHs from the 11th to the 30th day of incubation (for chrysene by 77.3 %, and for pyrene by 77.9 % of the initial content). Finally, after 360 days of the experiment, the content of fluorene accounted for 3.3 % of its initial content, anthracene – 1.2 %, pyrene 6.2 % and chrysene – 7.2 %.

The changes in the content of the PAHs investigated in the *Luvisol* sampled at Slesin (Lu7) are broken down in Fig. 4. At the first stage of the experiment, for the first 10 days, there was reported the highest decrease in the content of anthracene (65.8 % of the initial content), while as for fluorene, pyrene, and chrysene, the highest decrease was recorded 30 days after the start of the experiment, respectively by 70.6 %, 45.6 % and 45.1 % of their initial content. The amount of PAHs which remained non-

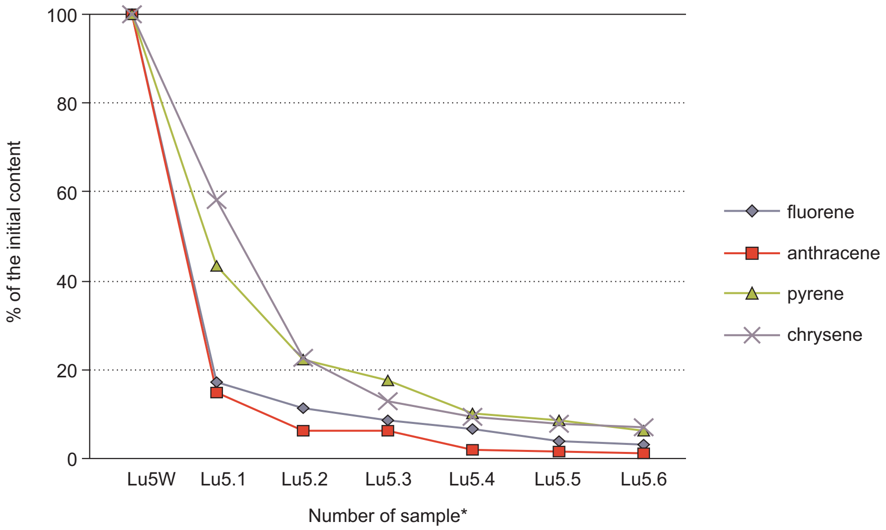

Fig. 3. Changes in the content of selected PAHs during incubation in the soil sampled at Orlinek (* – denotation as in Fig. 1)

-decomposed after 360 days of incubation was as follows: for anthracene – 2.3 % of the initial content, for fluorene – 3.6 %, for pyrene – 6.3 %, and for chrysene 8.6 % (Fig. 4).

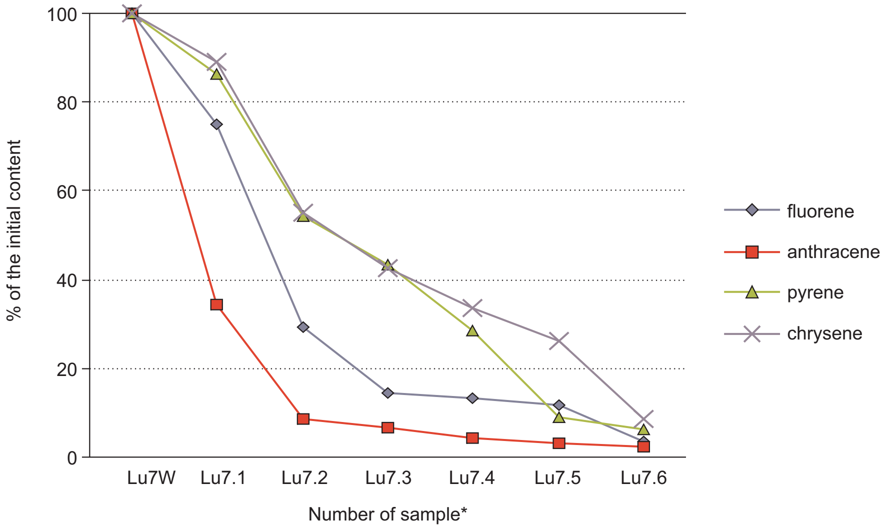

Fig. 4. Changes in the content of selected PAHs during incubation in the soil sampled at Slesin v/Bydgoszcz (* – denotation as in Fig. 1)

The content of the sum of the PAHs investigated in the *Luvisol* samples during the incubation is given in Table 3. After the first 10 days of the experiment the highest decrease in the content of the sum of PAHs was noted for soil sample Lu4 – 75.3 % of the initial content. The lowest decreases in the sum of PAHs (60.5 % and 66.6 %) were recorded for soil samples Lu3 and Lu5, respectively. As for soil sample Lu7, on the other hand, there was noted only a 28.4 % decrease in the contents of the sum of PAHs. Once the incubation was completed, the content of the sum of PAHs in the soil sampled at Bielawy and at Orlinek was higher than its initial content (Table 3). In the soil sampled at Slesin after 360 days, the content of the sum of PAHs was similar to its initial content.

Table 3

Content of the sum of PAHs during the incubation

Sample	Sum [μg/kg]			
	Lu3	Lu4	Lu5	Lu7
W	89.5	21.3	34.1	553
W’	10090	10021	10034	10553
10 days	3990	2479	3352	7558
30 days	2903	1671	1564	3928
60 days	2064	1315	1141	2849
120 days	1379	1090	715	2109
180 days	1032	684	543	1320
360 days	563	440	449	552

Table 4 presents the times of partial PAHs decomposition for two stages (0–30 days and 30–180 days) and for the entire incubation period (0–360 days).

Table 4

Partial decomposition time ($T_{1/2}$) – PAHs in soils at various experiment stages

PAHs	Period	Lu3	Lu4	Lu5	Lu7
Fluorene	0–30	12.63	9.04	9.59	17.00
	30–180	92.42	117.98	94.08	111.4
	0–360	76.47	71.46	73.16	75.04
Anthracene	0–30	8.23	8.15	7.54	8.45
	30–180	77.25	58.61	72.2	20.4
	0–360	56.9	55.18	56.86	66.27
Pyrene	0–30	19.73	15.74	13.76	34.16
	30–180	92.7	123.33	108.22	58.28
	0–360	100.2	90.27	89.59	90.24
Chrysene	0–30	32.6	13.79	14.04	34.68
	30–180	113.8	135.4	98.3	140
	0–360	103.39	94.28	94.72	101.78
Sum	0–30	16.69	11.61	11.19	21.04
	30–180	100.51	116.38	98.26	95.32
	0–360	86.44	79.82	80.30	84.55

At the initial stage there were noted the lowest values of the partial PAHs decomposition, which means that the transformations of hydrocarbons over that period were most intensive. The highest decomposition rate was recorded for anthracene; the lowest values of the partial decomposition time in the soil samples, whereas the lowest rate was noted for chrysene for which the highest $T_{1/2}$ values were recorded. Interestingly, over that period fluorene, anthracene, pyrene and chrysene were undergoing the slowest transformations in the case for the soil sample exposed to a direct effect of PAHs, sampled at Slesin (Lu7). Throughout the incubation period, similarly as over the first 30 days, the highest decomposition rate was noted for anthracene (the lowest values of parameter $T_{1/2}$).

Discussion

The content of PAHs in soils was determined by the sampling site. In the soils not exposed to a direct effect of PAHs there were noted low contents of those compounds (Table 2). Similar relations were reported by Adamczewska et al [8] and Zerbe et al [11]. Higher PAHs contents were recorded for the soil sampled in the close vicinity of the transport route, namely exposed to a direct effect of those compounds. A few-fold higher PAHs contents in soils exposed to intensive effects of transport coincide with the results reported by Adamczewska et al [8] and Kluska and Kroszczynski [9]. Weiss et al [13] and Wilcke and Amelung [19] report that high contents of such PAHs as anthracene, pyrene or chrysene point to an intensive human impact on the environment.

Interestingly, soil sample Lu3W sampled at the site exposed to a direct effect of PAHs showed lower PAHs content than soil sample Lu7W, also sampled at the site exposed to a direct effect of PAHs (Table 2), which demonstrates a moderate anthropopressure for soil Lu3W.

In all the soils investigated the highest decomposition of the compounds analysed occurred over the first 10 days of the experiment and then from the 11th to the 30th day of incubation (Figs 1–4). Similar relationships were reported by Maliszewska-Kordybach [35] demonstrating the highest PAHs losses in the first 30 days of soil sample incubation. According to Maliszewska-Kordybach [30, 33] and Maliszewska-Kordybach and Masiak [34], such high decreases in the PAHs content at the first decomposition stages are mostly due to microbiological processes. The reason of such a fast decomposition of the hydrocarbons analysed at the initial period of incubation by soil microorganisms are the experimental conditions: moisture: 50 % of field water capacity, temperature 20–25 ^{o}C. As reported by Bossert and Bartha [28] as well as Maliszewska-Kordybach [29], soil moisture ranging from 37 to 65 % of field water capacity creates optimal conditions for the development of soil microflora participating in the decomposition of PAHs. Whereas 15–38 ^{o}C is the temperature optimal for bacteria and fungi which take part in PAHs decomposition [30–32].

A fast decomposition at the initial stage of the experiment was observed for fluorene and anthracene, as compared with pyrene and chrysene, which is due to the fact that the rate of microbiological decomposition is higher for the compounds of a lower molecular weight and a lower number of aromatic rings. According to Maliszewska-Kordybach

[33] 3-ring fluorene and anthracene can be not only decomposed but also used by bacteria as the source of carbon and energy.

As for fluorene and anthracene, a high decrease in their content over the first 30 days of incubation can be due to abiotic transformations which are a result of chemical oxidation, photodegradation, sorption, oxidation and leaching [16, 33]. Interestingly, however, the conditions of the experiment made the photodegradation and leaching impossible. One can assume that in their case it was oxidation which triggered considerable soil loss mechanism, which is seen from the physicochemical properties of those compounds. Henry's constants for fluorene and anthracene fall within the range $10^{-5} < H < 10^{-3}$ atm/mol/m^3 classified as the range for compounds of moderate volatility [16].

A high stability of pyrene and chrysene in the soil samples is connected with their low susceptibility to oxidation, which is seen from a low vapour pressure and low solubility in water [2]. Other causes of high resistance of those compounds to the processes of decomposition can be found in their structure. The pattern of pyrene and chrysene rings shows a high thermodynamic stability [16]. A greater stability of 4-ring hydrocarbons is also due to their stronger sorption by organic matter which, as a result, limits the bioavailability of those PAHs. The lowest intensity of decomposition among the PAHs was recorded for chrysene, a compound of the highest molecular weight.

The coefficients of correlation recorded between the time of partial PAHs decomposition and the content of TOC, the content of C_{HAs} and C_{HUMIN} show that the intensity of decomposition of fluorene, anthracene and chrysene was also determined by soil properties (Table 5). The lowest decomposition rate for the first 30 days was found for soil sample Lu7 of the highest, of all the soils, content of organic carbon, carbon of the fraction of humic acids and humins (Table 1). The above relationships confirm the role played by organic matter in PAHs sorption processes. Many authors [3, 21, 22, 30] claim that PAHs sorption by the organic soil fraction is the basic process determining the activity and the bioavailability of hydrocarbons in the soil environment.

Table 5

Significant values of the coefficients of correlation between the time of partial PAHs decomposition and selected parameters defining soil properties

Parameter	Fluorene	Anthracene	Pyrene	Chrysene	Sum
	$T_{1/2}$ for 0–30 days				
TOC [g/kg]	0.749	—	—	0.757	0.711
C_{HAs} [g/kg]	0.946	—	—	0.868	0.911
C_{HUMIN} [g/kg]	0.723	—	—	0.799	0.708
$T_{1/2}$ for 0–360 days					
TOC [g/kg]	0.852	0.658	—	0.744	0.732
C_{HAs} [g/kg]	0.775	0.886	—	0.788	0.740
C_{HUMIN} [g/kg]	0.921	0.578	—	0.813	0.812

The PAHs stability investigated in all the *Luvisol* samples was increasing in the following order: chrysene > pyrene > fluorene > anthracene.

Conclusions

1. The contents of PAHs (fluorene, anthracene, pyrene and chrysene) in the samples of soils additionally unpolluted with PAHs depend on the sampling site. Higher PAHs contents were recorded for the soil sampled in a close vicinity of the transport route, as compared with the soil sampled away from the transport route.

2. The decomposition rate of PAHs introduced into soils depended on the experiment duration. The PAHs degradation process was most intensive for the first 30 days of incubation.

3. The PAHs decomposition intensity at the initial stage of incubation depended on the PAHs properties. A higher decomposition rate, as compared with pyrene and chrysene, was noted for anthracene and fluorene, as the compounds of a lower molecular weight and a lower number of aromatic rings.

4. The lowest PAHs decomposition rate was recorded for the samples of soil with the highest content of organic carbon, carbon of the fraction of humic acids and humins, which clearly points to an essential role of organic matter in PAHs sorption processes.

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SZYBKOŚĆ ROZKŁADU ANTRACENU, FLUORENU, PIRENU I CHRYZENU W GLEBACH PŁOWYCH

Katedra Chemii Środowiska, Wydział Rolnictwa i Biotechnologii
Uniwersytet Technologiczno-Przyrodniczy w Bydgoszczy

Abstrakt: Celem pracy było określenie trwałości i intensywności rozkładu wybranych wielopierścieniowych węglowodorów aromatycznych (fluorenu, antracenu, pirenu i chryzenu) w glebach płowych. Do badań pobrano próbki gleb reprezentatywne dla Regionu Kujawsko-Pomorskiego z terenów narażonych i nienarażonych bezpośrednio na zanieczyszczenia WWA. Próbki gleb zanieczyszczono wybranymi WWA w ilości odpowiadającej 10 mg WWA/kg. Zanieczyszczone WWA próbki gleb inkubowano 10, 30, 60, 120, 180 i 360 dni w temperaturze 20–25 ^{o}C i w stałej wilgotności – 50 % polowej pojemności wodnej. W celu oznaczenia zawartości WWA zastosowano metodykę wykorzystującą wysokosprawną chromatografię cieczową (HPLC). Stwierdzono, że najwięcej WWA rozkładało się w czasie pierwszych 30 dni prowadzenia doświadczenia, przy czym fluoren i antracen rozkładały się zdecydowanie szybciej niż piren i chryzen. Najwolniejszy rozkład WWA zachodził w próbkach gleby charakteryzującej się najwyższą zawartością węgla organicznego, węgla frakcji kwasów huminowych i humin, co w sposób jednoznaczny wskazuje na istotną rolę materii organicznej w procesach sorpcji WWA.

Słowa kluczowe: WWA, HPLC, gleby płowe

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Anna KWIECIŃSKA[1*] and Krystyna KONIECZNY[1]

APPLICATION OF MEMBRANE PROCESSES IN TREATMENT OF SLURRY FROM HIGH-DENSITY PIG FARMING

ZASTOSOWANIE PROCESÓW MEMBRANOWYCH W ZAGOSPODAROWANIU GNOJOWICY TRZODY CHLEWNEJ

Abstract: The aim of the study was to determine the effectiveness of integrated system: ultrafiltration and two-step reverse osmosis to recover water from pig slurry. The natural separation *ie* sedimentation and floatation were used to prepare feed for membrane treatment. Obtained supernatant was introduced to pilot scale installation for membrane filtration equipped with ceramic tubular UF membrane of pore size 5 nm. The cleaned stream was polished on polyamide RO membranes. The capacity of the process and the quality of cleaned streams were used to evaluate the effectiveness of the treatment. Washing of the ceramic membrane via back flushing with water and chemicals *ie* 0.5 % solutions of sodium hydroxide and nitric acid was also performed.

The study revealed that proposed system is suitable to recover water from pig slurry. The final product quality is suitable to reuse it on farms *eg* for washing animals, farmhouses or in heating/cooling systems. The advantage is also the concentration of organic compounds and nutrients in the form of retentate, which can be still used for fertilization or agricultural biogas production.

Keywords: pig slurry, ultrafiltration, reverse osmosis, fouling, water recovery

Introduction

The slurry is produced during non-bedding livestock farming on industrial farms. It is a liquid mixture of animal feces, urea and technological water [1, 2]. Slurry properties depend on many factors, among which number and age of animals, type of a feed, amount of water used for washing of farmhouses and animals, storage method, season and atmospheric conditions are the most important [3, 4].

Slurry management and utilization methods need to be developed and improved. Nowadays, slurry is mainly used for fertilizing purposes (*ca* 30 % of total production),

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[1] Division of Environmental Chemistry and Membrane Processes, Institute of Water and Wastewater Engineering, Faculty of Energy and Environmental Engineering, Silesian University of Technology, ul. S. Konarskiego 18, 44–100 Gliwice, Poland, phone: +48 32 237 29 81, fax. +48 32 237 10 47, email: anna.kwiecinska@polsl.pl

* Corresponding author

and, in much lower extent, for biogas and compost production. Different methods of slurry management are widely discussed in the literature. However, the attention is usually focused on nutrients recovery, *ie* nitrogen and phosphorus, or organic matter [5–7], while the possibility of water production from slurry is omitted. The amount of water in slurry can reach up to 99 %, thus it can be considered to treat it as a source of useable water. Such an assumption can be realized with the application of membrane processes, which are already widely used in water and wastewater treatment [8, 9]. Low-pressure membrane processes *ie* micro- and ultrafiltration practically guarantee the rejection of microorganisms, what results in production of sanitary safe fertilizing stream. Moreover, their combination with high-pressure membrane techniques *ie* nanofiltration and reverse osmosis can result in the recovery of water of useable quality [10–13].

The membrane filtration is mostly accompanied by the phenomenon called fouling *ie* the deposition of organic and inorganic impurities on the membrane surface and inside membrane pores. Fouling is the undesired process as it causes the decrease of the process capacity *ie* the permeate flux decreases in time. Generally, two forms of fouling can be distinguished. First is the reversible fouling caused by the deposition of the impurities on the membrane surface which leads to the formation of the filtration cake. Second one is the irreversible fouling which takes place when particles are deposited inside membrane pores. While the reversible fouling can be easily removed by simple flushing or backflushing of the membrane, the irreversible fouling requires more sophisticated methods of cleaning including chemical washing. However, even such aggressive methods are not always enough to completely recover the membrane capacity. In order to reduce the impact of fouling the preliminary treatment processes can be applied and/or filtration conditions optimization *ie* the transmembrane pressure the filtration mode can be made [14–16].

The aim and the methodology of the study

The aim of the presented study was to determine the effectiveness of integrated system comprised of ultrafiltration (UF) and two-step reverse osmosis (RO) to recover water from pig slurry. The natural separation processes *ie* sedimentation and floatation were used to prepare feed for the low-pressure membrane treatment. The obtained supernatant was introduced to the pilot scale installation for membrane filtration. The system was equipped with ceramic, tubular, ultrafiltration membrane of separation area 0.2 m^2 and pore size 5 nm (by Pall). The process was carried out at the pressure 0.3 MPa. The scheme of the installation is shown in Fig. 1.

The 12 dm^3 portion of the supernatant was introduced to the feed tank, and the process was carried out until 50 % of the initial volume was recovered in the form of the permeate. The time necessary to collect 0.2 dm^3 permeate was measured.

The cleaned stream *ie* UF permeate was next introduced to the laboratory membrane installation KMS Cell CF1 (by Koch) and polished on flat sheet, polyamide reverse osmosis membranes of effective separation area 28 cm^2 (by Koch). The polishing operation comprised of two-steps which were carried out at pressure 2.0 MPa. The scheme of the laboratory scale installation is shown in Fig. 2.

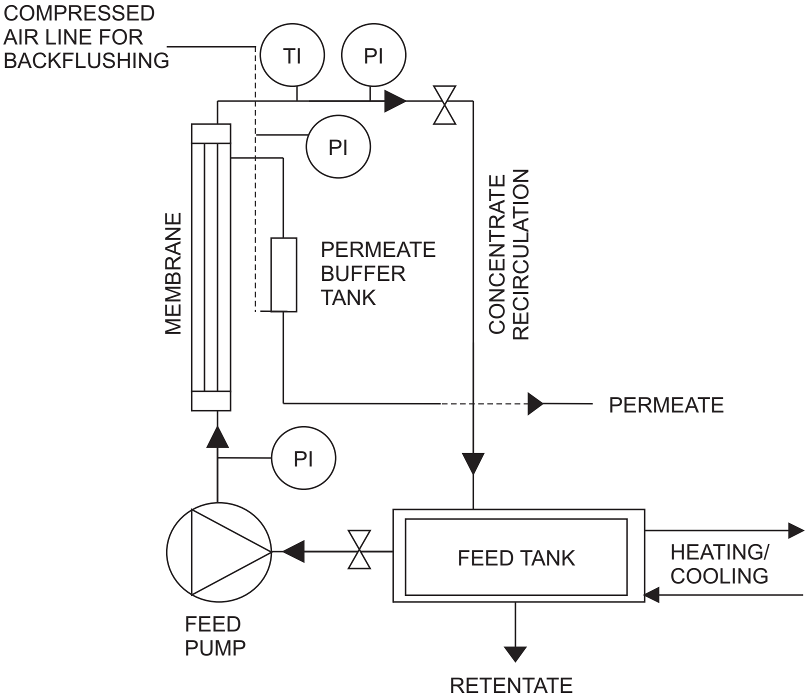

Fig. 1. The scheme of the pilot scale PALL installation for membrane filtration (TI – temperature indicator, PI – pressure indicator)

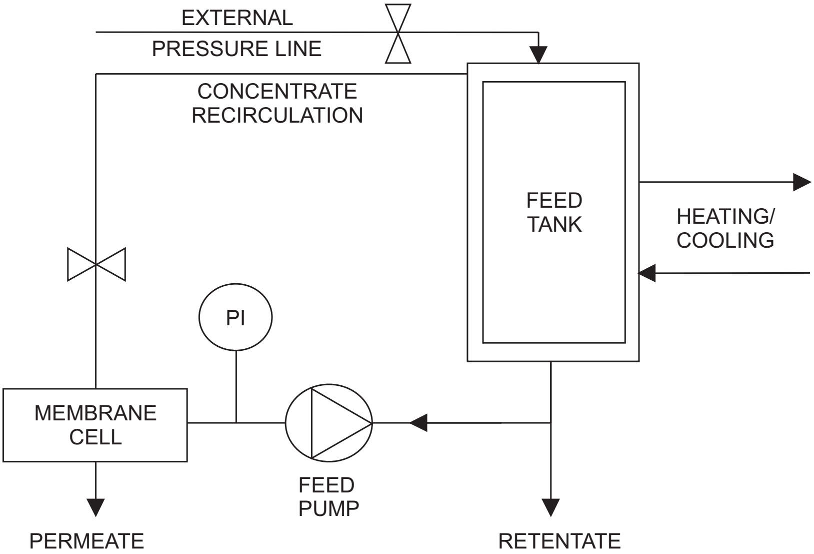

Fig. 2. The scheme of the laboratory scale KOCH installation for membrane filtration (PI – pressure indicator)

0.5 dm^3 of UF permeate was introduced to the 1st step RO and the process was carried out until 50 % of the initial volume was obtained in the form of the permeate. The time of the collection of 50 cm^3 permeate was measured. 50 cm^3 of the obtained filtrate was taken to analysis and the rest was polished via 2nd step RO. The process was also carried out until 50 % of the initial volume was recovered and the time of collection of 10 cm^3 permeate was measured. The overall ratio of the 2nd RO permeate volume to the crude slurry volume was equal to 12.5 %.

The capacity of the process and the quality of cleaned streams were used to evaluate the effectiveness of the treatment. Additionally, washing of the ceramic membrane was made. Firstly, the impact of the backflushing with the permeate during the process run on the filtration capacity was checked. Next, after the slurry treatment was finished, the membrane was washed via back flushing with water and chemicals *ie* 0.5 % solutions of sodium hydroxide and nitric acid. The impact of the cleaning method on the initial flux recovery was established.

Following parameters were determined in all process streams: pH, conductivity, dry mass, COD (*chemical oxygen demand*), TOC (*total organic carbon*), TC (*total carbon*), N_{tot} (*total nitrogen*), N-NH_4^+ (*ammonium nitrogen*), P-PO_4^{3-} (*phosphate phosphorus*), Cl^- (*chlorides*) and SO_4^{2-} (*sulphates*). COD, concentrations of PO_4^{3-}, N_{tot} and N-NH_4^+ were determined according to Hach Lange methodology. Concentrations of Cl^- and SO_4^{2-} were measured using ionic chromatograph DX 120 by Dionex. Particular forms of carbon were analyzed with the use of Multi N/C analyzer by Jena Analytic.

Results and discussion

In the first part the results of membrane characterization via determination of dependences of deionized water flux on pressure were presented. Next, the performance of slurry treatment was discussed including processes capacity, washing methods effectiveness and membrane fouling. Finally, product qualities and impurities retention at particular slurry treatment steps was shown.

Membranes characterization

The filtration of the slurry was preceded with the characterization of all membranes applied *ie* the determination of the dependence of deionized water flux on pressure. In case of ultrafiltration ceramic membrane the pressure was in the range of 0.1–0.3 MPa (with 0.05 MPa increase step), while in case of reverse osmosis membranes it was of 1.0–3.0 MPa (with 0.5 MPa increase step). The determined dependences are shown in Fig. 3a and 3b.

All determined dependences possessed linear character with the correlation coefficient almost equal to 1. Slight differences in fluxes for particular pressures observed in case of reverse osmosis membrane resulted of the irregular polymeric structure of the membrane.

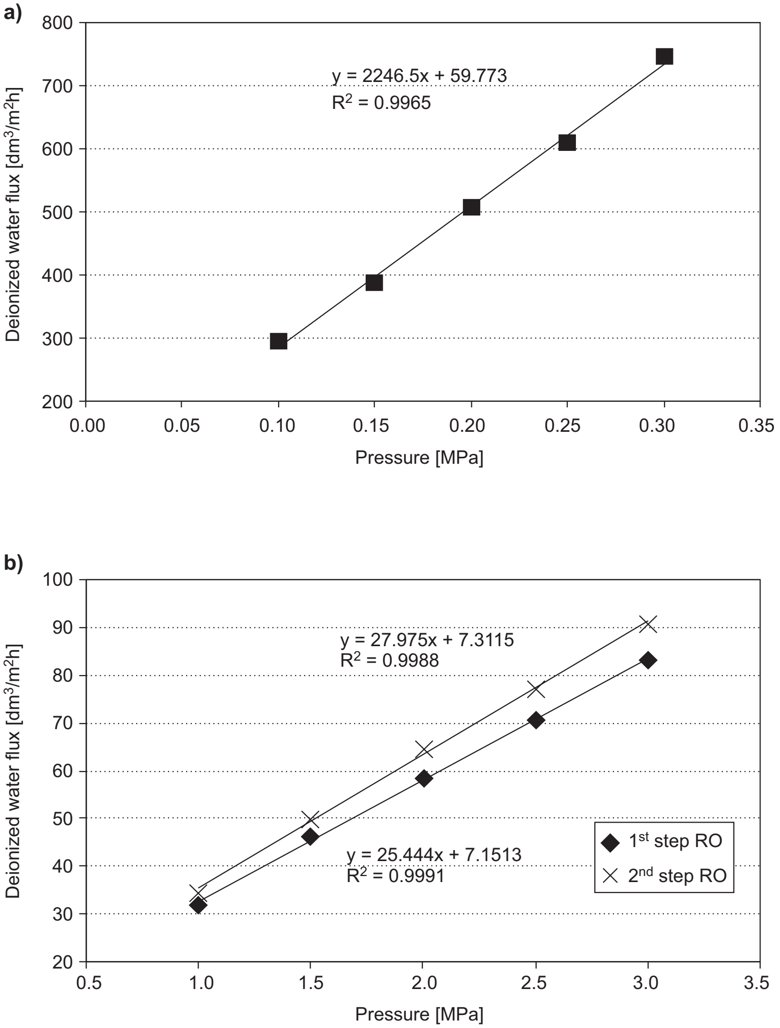

Fig. 3. The dependence of deionized water flux on pressure determined for: a) UF ceramic membrane, b) RO polyamide membranes

Slurry treatment

During the treatment of the slurry the decrease of permeate flux in time was observed for all performed filtrations. It indicated on the occurrence of fouling phenomenon, severness of which depended on the process. The change of the permeate flux observed during the processes run is shown in figures 4a–c.

The decrease of permeate flux observed for UF membrane was gentle. The backflushing of the membrane with the permeate performed in the 3rd hour of the

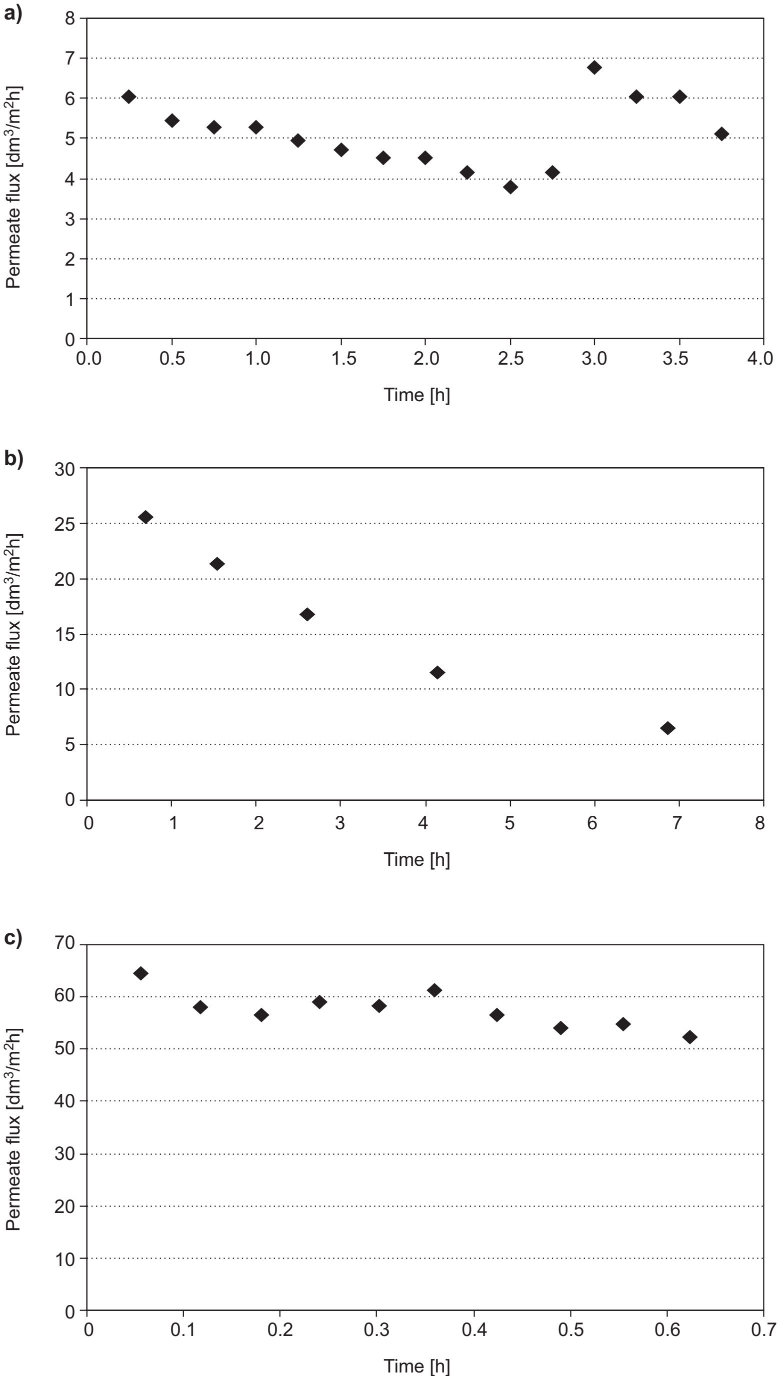

Fig. 4. Change of the permeate flux during: a) ultrafiltration, b) 1st step RO, c) 2nd step RO

process run allowed to increase the flux slightly above the initially measured value (Fig. 4a). However, it was found that the membrane blocking occurred at the beginning of the process and the impurities were deposited inside membrane pores.

The highest decrease of the process capacity in time was observed for the 1st step RO. The final permeate flux was 80 % lower than the initial one (Fig. 4b). However, oppositely as in case of the UF process, the main cause of the capacity decrease was the formation of the filtration cake during the process, thickness of which increased during the process run.

The permeate flux decrease observed during the final treatment was gentle, as in case of UF. The initial flux was comparable with one measured for distilled water, and the final one was found to be only 20 % lower (Fig. 4c).

In figures 5a–c the overall comparison of water and slurry fluxes measured for membranes (1) before the slurry treatment (deionized water) (2) during treatment (slurry) (3) after treatment (deionized water) (4) after chemical cleaning (deionized water – only for ceramic membrane) are shown. The unit of the fluxes for ultrafiltration was recalculated into unit pressure, as the measure of the water flux after backflushing with water and chemical cleaning was measured at 0.1 MPa, while the slurry filtration was carried out at 0.3 MPa.

It was observed that the filtration of slurry caused a significant decrease of UF membrane capacity (ratio of slurry flux to initial deionized water flux – 0.7 %) (Fig. 5a). The measurement of water flux after the treatment process preceded with 1-hour backflushing with water showed 12 % recovery of the initial value. It indicated on the irreversible character of membrane fouling caused mainly by the deposition of impurities in membrane pores. Thus, the membrane was cleaned chemically using 0.5 % solutions of first sodium hydroxide and followed by nitric acid. Every washing step was carried out for 15 minutes. It allowed to recover 70 % of the initial membrane capacity.

The filtration of permeate from UF also caused the decrease of 1st step RO membrane capacity (23 % of initial value) (Fig. 5b). However, simple flushing of membrane with water enabled the total capacity recovery. Moreover, the measured water flux after the process was higher than the initial one. It could have been caused by several reasons. Firstly, only the reversible membrane fouling occurred and impurities were deposited on membrane surface forming washable filtration cake. Secondly, adsorption of some compounds (both, organic and inorganic) could have modified membrane surface increasing the hydrophilicity of membrane material. Thirdly, the diffusion of compounds through the membrane could loosen its polymeric structure.

The final polishing of treated slurry via 2nd step RO caused the lowest decrease of membrane capacity (83 % of initial value).The flushing of membrane in water enabled almost 98 % of initial capacity recovery (Fig. 5c). In case of this membrane, water flux after the process is slightly lower than at the beginning. As higher molecular weight compounds compound were already removed during the 1st step RO treatment both, the formation of protective filtration cake layer was limited and smaller particles did not affect membrane structure. Thus, slight irreversible membrane fouling occurred.

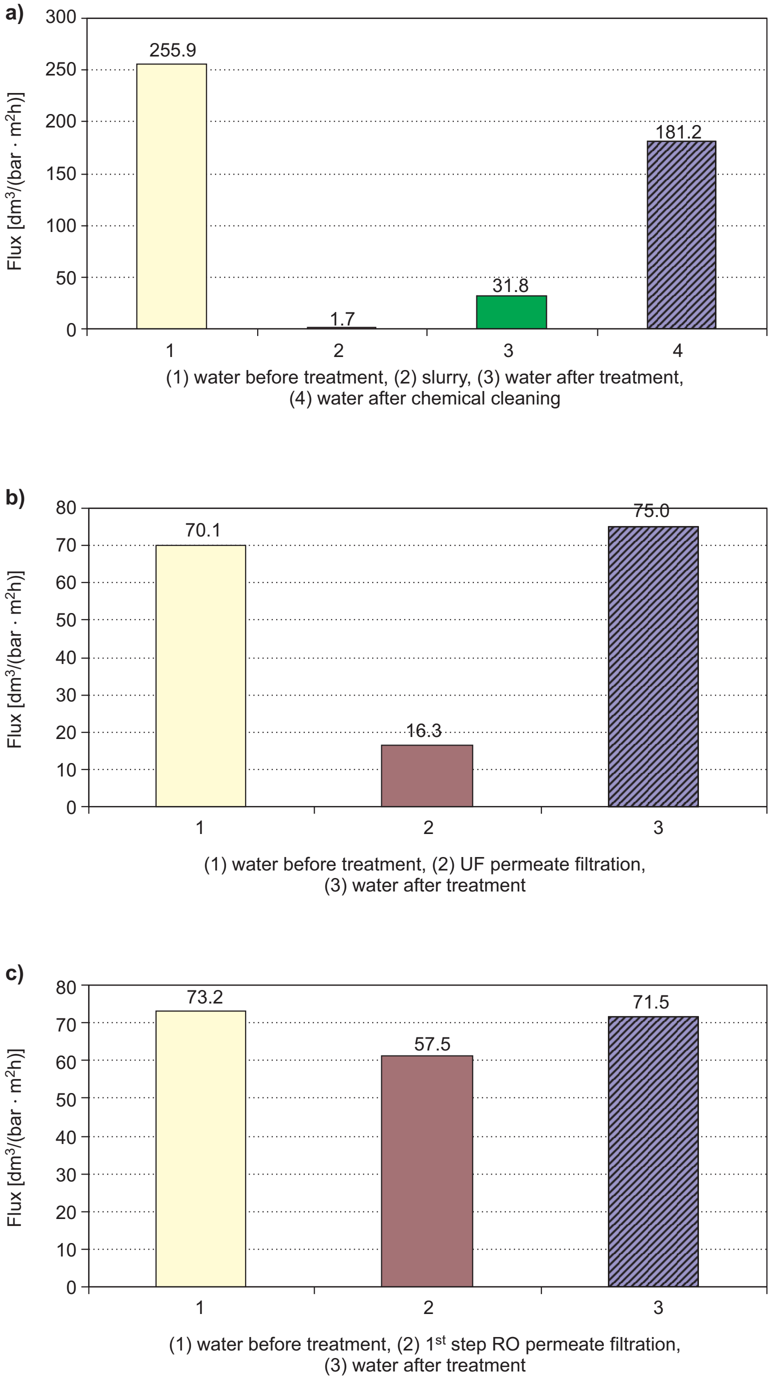

Fig. 5. Fluxes measured for membranes: a) ceramic UF, b) polyamide 1st step RO, c) polyamide 2nd step RO

Quality of process streams

In Table 1 the comparison of crude slurry, supernatant and cleaned process streams including retention rates is shown.

Table 1

Comparison of process streams parameters

Parameter	Unit	CS	SN	R [%]	UF P	R [%]	RO1 P	R [%]	RO2 P	R [%]
pH	—	5.98	6.08		6.37		6.47		8.65	
Cond.	mS/cm	18.8	18.1	4	16.3	10	1.6	90	0.062	96
COD	g/dm^3	38	29	23	18	39	0.953	95	< 5	99
$N\text{-}NH_4$	mg/dm3	2095	1961	6	1490	24	126	92	7.8	94
N_{tot}	mg/dm^3	2550	2367	7	1560	34	178	89	9	95
$P\text{-}PO_4$	mg/dm3	695	578	17	354	39	5.2	99	0	100
Cl^-	mg/dm^3	1022	1004	2	951	5	164	83	2	99
PO_4^{3-}	mg/dm^3	2274	1894	17	1217	36	15	99	0	100
SO_4^{2-}	mg/dm^3	300	294	2	284	3	8	97	0	100
TC	mg/dm^3	11871	8904	25	4390	51	227	95	4.24	98
TOC	mg/dm^3	11508	8779	24	3821	56	202	95	2.1	99
d.m.	%	4.2	1.4	67	0.7	51	n/a	n/a	n/a	n/a

CS – crude slurry, SN – supernatant, P – permeate, R – retention rate, n/a – not analyzed, d.m. – dry matter.

It was found that natural separation processes *ie* sedimentation and flotation already caused the reduction of COD, phosphates and carbon concentrations. Ultrafiltration influenced mainly on organic compounds and phosphates content. Besides, the decrease in ammonium nitrogen was observed what was related to the emission of the compound during the treatment. The rates of retention obtained for 1st RO step were in the range from 83 % (Cl^-) to 99 % ($P\text{-}PO_4$). Despite such high impurities rejection the quality of the permeate was still poor, hence it was decided to polish it via 2nd RO step. The retention rates obtained in the finial treatment process varied form 94 % ($N\text{-}NH_4$) to 100 %. The final product quality was found to be satisfactory considering its further use on farms for *ie* washing of animals and farmhouses, fields irrigation or heating/cooling purposes.

Conclusions

The study discussed the possibility of application of integrated membrane processes *ie* ultrafiltration/two step reverse osmosis for recovery of water from pig slurry. It was found that the proposed system was suitable for that purpose. The final product was good quality water that could be successfully reused on farms. The ratio of 2nd RO step permeate volume to crude slurry volume was equal to 15 %.

The highest decrease in membranes capacity was observed for UF membrane, next for 2nd RO and finally for 1st step RO. The fouling of UF membranes was significant and irreversible *ie* washing of membrane via backflushing with water did not improve significantly its capacity. The cleaning of membrane with chemicals *ie* 0.5 % solutions of NaOH and HNO_3 enabled to recover membrane capacity up to 70 % of the initial one. In both RO processes the decrease of membranes capacity was observed, however simple flushing of membranes with water enabled the total recovery of the initial water flux. Moreover, in the 1st RO process the increase of capacity was observed. It could have been caused by several reasons *ie* the occurance of only reversible membrane fouling occurred, adsorption of some compounds (both, organic and inorganic) that could have modified membrane surface increasing the hydrophilicity of membrane material or the diffusion of compounds through the membrane could loosen its polymeric structure.

Acknowledgements

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ZASTOSOWANIE PROCESÓW MEMBRANOWYCH W ZAGOSPODAROWANIU GNOJOWICY TRZODY CHLEWNEJ

Zakład Chemii Środowiska i Procesów Membranowych
Politechnika Śląska w Gliwicach

Abstrakt: Celem przeprowadzonych badań było określenie efektywności zintegrowanego system złożonego z ultrafiltracji i dwustopniowej odwróconej osmozy do odzysku wody z gnojowicy trzody chlewnej.

W przygotowaniu nadawy do filtracji membranowej wykorzystano naturalnie zachodzące procesy separacji, tj. sedymentację i flotację. Otrzymaną w ten sposób ciecz nadosadową wprowadzono na instalację pilotową wyposażoną w ceramiczne rurowe membrany ultrafiltracyjne o średnicy porów 5 nm. Otrzymany strumień permeatu doczyszczano na poliamidowych membranach osmotycznych. Do oceny efektywności zastosowanego systemu wykorzystano wydajność procesów filtracji membranowej oraz jakość oczyszczanych strumieni. Dodatkowo, w przypadku membran ceramicznych zbadano wpływ mycia wstecznego wodą oraz środkami chemicznymi, tj. 0,5 % roztworami wodorotlenku sodu oraz kwasu azotowego na przywrócenie początkowej wydajności membran.

Badania wykazały, że proponowany system oczyszczania może zostać wykorzystany do odzysku wody z gnojowicy trzody chlewnej. Jakość końcowego produktu pozwala na jego ponowne wykorzystanie na farmie do np. mycia zwierząt i obiektów hodowlanych czy też w systemach chłodząco/grzewczych. Dodatkową zaletą procesu jest zatężenie związków nawozowych i organicznych w postaci retentate, który może zostać wykorzystany do celów nawozowych lub w produkcji biogazu rolniczego.

Słowa kluczowe: gnojowica trzody chlewnej, ultrafiltracja, odwrócona osmoza, fouling, odzysk wody

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Andrzej GREINERT[1*], Róza FRUZINSKA,
Jakub KOSTECKI and Karolina BEDNARZ[2]

POSSIBILITIES OF HEAVY METALS AVAILABLE FOR PLANTS DETERMINATION IN THE SOIL OF AN INDUSTRIAL ZONE

MOŻLIWOŚCI OZNACZANIA METALI CIĘŻKICH DOSTĘPNYCH DLA ROŚLIN W GLEBACH TERENÓW PRZEMYSŁOWYCH

Abstract: For the determination of bioavailable form of heavy metals in soils the extraction methods using different extractors have been used in soil science. They presented analyzed content in the soil solution and different ways combined with the sorption complex. The problem is to indicate the boundary beyond which the element can be regarded as permanently absorbed. This problem is even larger in relation to soil under strong anthropopressure, which sorption complex is subjected to many actions dynamic sliding the boundary between sorption and desorption. The work shows the results of analyses made on the industrial soil material (Technosols), using as extractors 1 M salt solutions: KCl, $MgCl_2$, NH_4NO_3, KNO_3, NH_4OAc and 0.01 M $CaCl_2$, to demonstrate the potential bioavailability of Cd, Co, Cu, Ni, Pb and Zn. 1 M KNO_3 and KCl solutions were relatively stronger extractors of Ni, Cd and Co than the other ones. The relatively weak extractors of analysed heavy metals are 0.01 M $CaCl_2$, 1 M $MgCl_2$ and 1 M NH_4NO_3. An intermediate effect of 1 M NH_4OAc extraction has been marked.

Keywords: heavy metals extraction, available form of heavy metals, industrial soil

Introduction

The content of heavy metals in soils is showing a strong dependance from the form of land use and development direction of the state (impact of the pro-environmental or pro-industrial regulations). In the legal systems of most countries of the world [1, 2], and also in modern scientific considerations [3–6] a variation of heavy metals limits for protected nature areas, forests and parks, agricultural, urban, industrial and communications has been presented. Urban areas are often additionally divided on: residential areas, children playgrounds, parks, gardens, grounds services, manufacturing and others.

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[1] Department of Land Protection and Reclamation, Institute of Environmental Engineering, University of Zielona Gora, ul. Prof. Z. Szafrana 15, 65–516 Zielona Góra, Poland, phone: +48 68 328 26 80, email: A.Greinert@iis.uz.zgora.pl, R.Fruzinska@iis.uz.zgora.pl, J.Kostecki@iis.uz.zgora.pl

[2] Student graduated in Institute of Environmental Engineering, University of Zielona Gora.

* Corresponding author.

The elements described in this study are presented in the environment as a micronutrients essential for living organisms (cobalt, copper, nickel, zinc), as well as elements of unknown physiological role (cadmium, lead) – [7]. Microelements occurring in soils in trace amounts are not environmental problem – on the contrary, the problem may be linked with their lack or limited bioavailability to plants. Otherwise this issue is presented within the industrial, urban, transport and the post-mining areas. Many authors have reported to them the problem of the oversized heavy metal content in the superficial layers of soils, directly threatening organisms, as well as indirectly – through the contamination of groundwaters, surface waters (the effect of infiltration and erosion) and air (the effect of the secondary secondary dust generation) – [8, 9].

If the case of necessity of the oversized heavy metals content determination exists, it appears the problem to indicate the state (form) of heavy metals in the given soil situation. The use of specific extractors, as well as their concentrations, operating conditions and time to reflect the existing and possible mobility of heavy metals in soil and aquatic environments is a commonly discussed problem [7, 10]. This is particularly important to form readily solved in water and poorly sorbed by the soil sorption complex (exchangeable form). Additional difficulty is the inconsistent behavior of mineral parts in soils of different mechanical composition, pH, salinity, organic matter content, redox potential and other characteristics [11, 12]. Widely discussed are differences in sorption and desorption phenomena in soils of urban and industrial areas showing the degradation and the presence of many different impurities – mainly of a waste origin [13–16].

The occurrence of various chemical and physical forms of the element in the analysed material is called speciation, and the identification and quantification is the subject of speciation analysis. The term is also used for naming of a variety analysis and extraction procedures. In the studies used for the speciation analysis of heavy metals in soils are used two basic types of proceedings [12]:

– single extraction – using a solution simulating the natural conditions of components transition from soil to water;

– sequential extraction – which consists of a raw of extraction procedures with solutions of increasing activity, aimed to capture different forms of components binding in the soil.

For the determination of bioavailable heavy metals form and assessment of migration ability of metals associated with the solid phase the single extraction in the analysis of soils, sewage sludge, as well as solid wastes is often used. The single extraction technique is implemented using different extractants: non-buffered salt solutions, such as $MgCl_2$, $CaCl_2$, $NaNO_3$, NH_4NO_3, buffered solutions as $NH_4OAc/AcOH$, as well as complexing compounds. In literature it can be found the information about a good reflection of this technique to the natural phenomena occurring under the influence of rainfall and floods of water [10, 17]. While, they are found different procedures for heavy metals determination in the bioavailable or potentially bioavailable forms (exchangeable / non-specifically sorbed fraction), different in respect of used extractor, its concentration, method and time of soil samples mixing with the extractor and the pH of extraction environment [10, 18].

Aim of this study is to compare the efficiency of Cd, Co, Cu, Ni, Pb and Zn extraction from soil samples of industrial soils (Technosols) using a single extraction technique with 1 M solutions of salts: KCl, $MgCl_2$, NH_4NO_3, KNO_3, NH_4OAc and 0.01 M solution of $CaCl_2$.

Research object description

Soil samples were taken from the area of metal industry located in Zielona Gora (western Poland), occupying an area of approximately 11.5 hectares. Industrial activities on this area began in 1876 on the construction of wooden wagons. Successively dealt with producing agricultural machinery, steel structures of industrial halls, bridges and railway stations. In 1886, the construction of railway freight wagons and passenger cars, tanks, mail wagons, refrigerated, etc. has been started During the Second World War the factory produced vehicles and equipment for the army. There have been produced armored trains, canon parts, military vehicles, submarine hulls, aircraft components. After 1945, the plant named Zastal, dealt with the production of rolling stock, freight cars, diesel locomotives and steel structures [19, 20]. At the beginning of the twenty-first century, on the described site is going production of rolling stock and steel structures.

Materials and methods

Soil samples preparation

According to IUSS WG WRB classification [21], the soils covering the area being under investigation represent a taxon: Urbic Transportic Toxic Technosols. Soil samples were taken as a aggregate ones, collected from the 0–20 cm layer. Each sample consisted of material mixed from the 30 individual sampling points. They have been marked as: Z1, Z2, Z3. Z1 and Z2 samples were taken from areas adjacent to production facilities, while the Z3 from internal parking hardened with slag and rubble. The soil samples were air dried (35 ^{o}C), after that passed through the 2-mm sieve.

Laboratory and statistical methods

All studies were conducted in summer, 2010. Mechanical composition was determined for the parts below 2 mm using areometric method.The pH-H_2O and pH-1 M KCl values were measured with a glass electrode in the supernatant of a 1 : 2.5 soil : water suspension. EC was determined using conductometer EUTECH Instruments CyberScan in saturated paste. Basic sorption properties have been determined using the Kappen method for hydrolytic acidity (HA) and Pallmann method for bases content (BC). CEC was calculated by summarising the HA and BC. BS was calculated as a share of BC in the CEC and given in percent. Organic matter was determined by loss of ignition in a heating furnace at 550 ^{o}C.

For the analysis of heavy metals content from the aggregate sample was weighed 50 g of soil to each from the 18 plastic bottles; to every 3 bottles form this set 100 cm^3 of extractor was added:

- 1 M CH_3COONH_4 exchangeable form acc. to [22],
- 1 M KCl exchangeable form acc. to [23],
- 1 M KNO_3 exchangeable form acc. to [23],
- 1 M $MgCl_2$ exchangeable form acc. to [24],
- 0.01 M $CaCl_2$ exchangeable form acc. to [25, 26],
- 1 M NH_4NO_3 exchangeable form acc. to [25, 27].

For every combination the soil:solution ratio of 1 : 2 has been used. Bottles have been corked and placed in a rotating laboratory stirrer, leaving them for 1 hour of stirring at 25 ^{o}C [24]; stirrer speed: 60 times per minute. After that the suspension was filtered through medium paper filters to conical flasks, then poured into glass bottles. Markings have been made using a Varian atomic absorption spectrometer – FAAS method.

To ensure the accuracy and precision of the analysis, reagent blank and analytical duplicates were used. Three replicates have been used for each of the analytical procedures.

The results have been compared among themselves and also have been shown on the background of the subtotal content marked in aqua regia according to ISO 11466 [28].

The results were statistically analysed using Statsoft Statistica 9.1a for Windows procedures.

Results

Chosen physico-chemical characteristics of the soils

Samples from the industrial area were characterized by grain size of sand, which is typical for the area of Zielona Gora city. Admixtures of various waste materials and dust fallout from the lignite combustion have caused the effect of an alkaline pH and high total carbon content. Through the high water permeability of soils, the relatively low electrical conductivity has been recorded. Sorption properties are high due to about eight percent of the carbon content. From the data of Table 1 it is visible the uniform of basic physico-chemical properties of the described soils.

Table 1

Basic physico-chemical characteristics of the soils used in the experiment (mean values)

Soil sample	Mechanical composition of parts < 2 mm			TC [%]	pH		EC	HA	BC	CEC	BS
	Sand [%]	Silt [%]	Clay [%]		in H_2O	in 1 M KCl	[mS · cm^{-1}]	[cmol$_c$ · kg^{-1}]			[%]
Z1	92	8	0	8.1	7.77	7.53	0.1178	1.35	43.10	56.60	76.14
Z2	94	6	0	8.1	8.04	7.87	0.1027	0.75	46.10	53.60	86.00
Z3	92	8	0	8.2	8.02	7.78	0.1056	0.82	43.90	52.15	84.18

Heavy metals extraction

As a result of a single extraction of soil samples has been observed the low content of Cd, Co, Cu, Ni and Pb for 1 M NH_4NO_3 and 0.01 M $CaCl_2$. The lower results were obtained also with Zn extraction performed using 0.01 M $CaCl_2$. High content of Cd, Co, Cu, Ni and Pb were recorded when using for extraction 1 M KCl and 1 M KNO_3. For Zn, the highest content was noted in the 1 M NH_4OAc and 1 M $MgCl_2$ solutions. In the sample Z3 a relatively high content of Zn in the 1 M NH_4NO_3 extract has been also found.

Table 2

Results of heavy metals extraction from soils (mean content ± std. dev.)

Soil sample	Extractant	Cd	Co	Cu	Ni	Pb	Zn
		[mg · kg^{-1}]					
Z1	NH_4OAc	0.78 ± 0.02	6.43 ± 0.13	4.39 ± 0.36	1.56 ± 0.05	7.31 ± 0.17	137.6 ± 14.4
	KCl	2.31 ± 0.04	31.38 ± 0.66	7.09 ± 0.47	10.57 ± 0.06	24.40 ± 0.67	27.0 ± 0.5
	$MgCl_2$	0.55 ± 0.02	1.12 ± 0.04	0.59 ± 0.03	1.22 ± 0.28	3.20 ± 0.33	34.9 ± 2.2
	KNO_3	2.09 ± 0.15	14.10 ± 0.53	7.79 ± 0.26	8.73 ± 0.68	18.71 ± 1.99	15.7 ± 3.6
	NH_4NO_3	0.19 ± 0.02	0.74 ± 0.01	0.28 ± 0.02	0.99 ± 0.01	0.65 ± 0.23	15.8 ± 0.2
	$CaCl_2$	0.22 ± 0.02	0.58 ± 0.05	not det.	0.37 ± 0.09	1.32 ± 0.63	10.4 ± 0.3
Z2	NH_4OAc	0.82 ± 0.02	7.30 ± 0.10	8.41 ± 0.21	2.13 ± 0.25	6.25 ± 0.31	197.7 ± 11.3
	KCl	2.35 ± 0.15	30.47 ± 0.62	8.43 ± 0.21	9.35 ± 0.12	23.57 ± 1.32	29.3 ± 1.8
	$MgCl_2$	0.47 ± 0.02	1.23 ± 0.06	1.27 ± 0.23	1.67 ± 0.14	2.55 ± 0.24	31.3 ± 1.0
	KNO_3	2.85 ± 0.28	15.59 ± 0.21	10.18 ± 0.19	10.85 ± 3.24	22.47 ± 3.68	18.5 ± 1.9
	NH_4NO_3	0.27 ± 0.05	0.95 ± 0.05	1.07 ± 0.14	1.39 ± 0.16	1.52 ± 0.15	19.0 ± 0.5
	$CaCl_2$	0.33 ± 0.06	0.83 ± 0.04	0.13 ± 0.04	0.87 ± 0.02	0.81 ± 0.03	14.2 ± 1.1
Z3	NH_4OAc	1.29 ± 0.01	7.95 ± 0.19	11.37 ± 0.62	7.05 ± 0.3	6.45 ± 0.57	396.5 ± 3.3
	KCl	2.80 ± 0.03	25.60 ± 1.68	9.41 ± 0.12	12.73 ± 0.9	31.70 ± 0.88	37.5 ± 2.5
	$MgCl_2$	0.66 ± 0.05	1.60 ± 0.02	2.01 ± 0.24	1.66 ± 0.36	4.61 ± 0.48	239.4 ± 7.1
	KNO_3	3.51 ± 0.14	14.15 ± 1.07	10.32 ± 0.15	8.93 ± 1.36	21.15 ± 2.14	18.4 ± 3.2
	NH_4NO_3	0.26 ± 0.03	1.17 ± 0.05	3.51 ± 0.34	0.91 ± 0.08	4.55 ± 0.15	147.6 ± 14.9
	$CaCl_2$	0.26 ± 0.01	1.13 ± 0.05	0.09 ± 0.01	2.03 ± 0.05	2.45 ± 0.51	25.9 ± 4.0

Designation the analyzed subtotal heavy metals form showed the presence of [mg · kg^{-1}]:

Z1: Cd – 4.90, Co – 8.89, Cu – 58, Ni – 17.43, Pb – 146, Zn – 366;

Z2: Cd – 10.90, Co – 9.68, Cu – 115, Ni – 18.30, Pb – 128, Zn – 432;

Z3: Cd – 14.20, Co – 14.77, Cu – 366, Ni – 44.71, Pb – 714, Zn – 2713.

Referring the data obtained from the analysis of heavy metals content in the extracts described in Table 1 to the subtotal content (Table 3), it must be noted relatively high efficiency of 1 M KCl and 1 M KNO_3 to the extraction of Cd, Ni and Pb. 1 M KCl was also an effective extractor of Co. For this metal 1 M KNO_3 has been less effective extractor than 1 M NH_4OAc. With respect to Cu, 1 M KCl, 1 M KNO_3, as well as 1 M NH_4OAc proved to be more effective extractors than other ones. However, there was internal divergence between these three solutions compared to each of the analyzed samples. In the case of zinc the highest effectiveness of 1 M NH_4OAc has been evidently proved.

Table 3

Extraction efficiency of analysed chemical solutions compared to the subtotal content

Soil sample	Extractant	Cd	Co	Cu	Ni	Pb	Zn
		[%]					
Z1	NH_4OAc	15.9	72.3	7.6	9.0	5.0	37.6
	KCl	47.1	35.3	12.2	60.6	16.7	7.4
	$MgCl_2$	11.2	12.6	1.0	7.0	2.2	9.5
	KNO_3	42.7	15.9	13.4	50.1	12.8	4.3
	NH_4NO_3	3.9	8.3	0.5	5.7	0.4	4.3
	$CaCl_2$	4.5	6.5	0.0	2.1	0.9	2.8
Z2	NH_4OAc	7.5	75.4	7.3	11.6	4.9	45.8
	KCl	21.6	31.5	7.3	51.1	18.4	6.8
	$MgCl_2$	4.3	12.7	1.1	9.1	2.0	7.2
	KNO_3	26.1	16.5	8.9	59.3	17.6	4.3
	NH_4NO_3	2.5	9.8	0.9	7.6	1.2	4.4
	$CaCl_2$	3.0	8.6	0.1	4.8	0.6	3.3
Z3	NH_4OAc	9.1	53.8	3.1	15.8	0.9	14.6
	KCl	19.7	17.3	2.6	28.5	4.4	1.4
	$MgCl_2$	4.6	10.8	0.5	3.7	0.6	8.8
	KNO_3	24.7	9.5	2.8	20.0	3.0	0.7
	NH_4NO_3	1.8	7.9	1.0	2.0	0.6	5.4
	$CaCl_2$	1.8	7.7	0.0	4.5	0.3	1.0

For each extractor can be determined the effectiveness array:

1 M NH_4OAc	Co > Zn > Ni > Cd > Cu > Pb;
1 M KCl	Ni > Cd > Co > Pb > Cu > Zn;
1 M $MgCl_2$	Co > Zn > Cd > Ni > Pb > Cu;
1 M KNO_3	Ni > Cd > Co > Pb > Cu > Zn;
1 M NH_4NO_3	Co > Ni > Zn > Cd > Cu = Pb;
0.01 M $CaCl_2$	Co > Ni > Cd > Zn > Pb > Cu.

It can be indicated a number of similarities in setting of the analysed heavy metals extraction effectiveness arrays for the pairs of solutions: 1 M KCl and 1 M KNO_3, 1 M NH_4OAc and 1 M $MgCl_2$, 1 M NH_4NO_3 and 0.01 M $CaCl_2$.

Discussion

The difficulty of determining the bioavailable form of heavy metals in soils consists of diverse physiological responses of plants to the content of these elements. as well as the dynamics of changes in the sorption complex of soil and soil solution. Therefore. in many cases the authors do not use the pure concept of bioavailability. but potential bioavailability of elements. This means that under certain environmental conditions (*eg* acidification. shifting the balance between the processes of oxidation and reduction) part of the metals sorbed in the soil sorption complex can be desorbed and found in the soil solution. We come here to some element of risk analysis – how often can such conditions happens and on what scale? In the environmental analysis is often thought. that even the probability of one occurrence of such circumstances is enough for suggesting potential availability of elements.

Basta et al [29] and Rao et al [10] are suggesting caution in estimating the uptake of heavy metals by plants determined in soils using single extraction. This is due to the complex physiological conditions. This indicates a better procedure involving the use of the term of the potential availability of heavy metals for organisms. This problem does not resolve to adopt a different technique for determining the elements described in the soils. Sequential extraction method is fraught with the same dilemma.

The use of different extractors to perform into the liquid phase salts of heavy metals contained in the soil gives a different effect on the results of their content. Hence the need for a detailed description of not only the form of metal in the soil. but also the methodology of its extraction. Rao et al [10] are showing a huge variety of procedures available for the determination of bioavailable form of heavy metals in soils.

0.01 M $CaCl_2$ is extractant recommended in the Netherlands for heavy metals available for plants form evaluation [30]. Menzies et al [17] pointed out this extractor as well reflecting the phytoavailability of Cd. Ni and Zn. In light of the reported research in this paper should be noted. that this solution has demonstrated the effectiveness of some 7 % of subtotal Co content and about 3 % of subtotal Cd. Ni and Zn content. In other cases. the values were extremely low. Low scores of Cu extraction using calcium chloride solution (≤ 0.4 %) have been described by Schramel et al [25]. Sahuquillo et al [31] have found that for the most of heavy metals 0.01 M $CaCl_2$ extract no more than 1 % of their total form. but for Cd and Zn can be noted higher results (even up to 40 % Cd and 12 % Zn). The presented study did not support this thesis.

1 M $MgCl_2$ solution, proposed by Navas and Lindhorfer [32]. proved in the research effectiveness to Cd, C, Ni and Zn extraction. Significantly lower scores have been noted for Cu and Pb.

1 M NH_4NO_3 is a standard extractant for the described in the paper heavy metal form in Germany [33]. The studies have shown the ability to release more than 5 % of the subtotal Co and Ni form wits content determination. this solution. For other metals the extraction potential of described solution was low. Low scores of Cu extraction using calcium chloride solution (≤ 0.4 %) have been described by Schramel et al [25].

1 M KNO_3 can be used for non-specific sorbed (exchangeable) heavy metals content determination [18].

NH_4OAc is a salt of a weak base. According to Schramel et al [25] this compound formed metal acetate complexes. what tends to prevent readsorption of released cations. Menzies et al have pointed out NH4OAc as well reflecting phytoavailability of Cd [17]. In view of the presented work this extractor has mobilised 7.5–15.9 % of subtotal Cd. which indeed seems to confirm this thesis. Generally. described extractor can be characterised as balanced one in terms of extraction force for the analyzed elements.

except cobalt. Using of 1 M NH_4OAc for Co extraction may indicate excessively high mobility of this metal.

1 M KCl solution has been described by Grove et al [23] as similar in action with 1 M NH_4OAc. As has been shown for the situation described in this paper. the only similarity between 1 M KCl and NH_4OAc can be identified in the extraction of Cu. Other heavy metals were extracted with different intensity. although in both cases with high effectiveness in compare to the other analysed solutions.

According to Szumska and Gworek [18]. using the chloride salt as heavy metal extractors gives higher scores than in the case of nitrate salts use by the same cation and solution concentration. The examined relationship 1 M KNO_3 to 1 M KCl confirm this thesis in case of Co (mean 28.1 % vs. 14.0 % of subtotal form). Ni (mean 46.7 % vs. 43.1 %). Pb (mean 13.2 % vs. 11.1 %) and Zn (mean 5.2 % vs. 3.1 %). With respect to Cd and Cu the solutions of described salts behave similarly in terms of extraction efficiency – respectively (average values): 29.5 % vs. 31.2 % and 7.4 % vs. 8.4 %.

Conclusions

Many authors suggest the need for sequential extraction use. as giving more detailed information on the metal bind form in soil. They describe the single extraction method as not enough selective. It is difficult do not agree with these opinions. however. noting that the phenomena occurring in nature are generally less selective. For the practice turns out to be important to estimate the size of the possible release of metal into the soil solution under different external conditions and different properties of soils. Different extractors make it possible to desorbe of various amounts of metallic ions. KNO_3 and KCl are relatively stronger extractors of Ni. Cd and Co in relation to the other extractors. Relatively weak extractors of the analyzed heavy metals are: $CaCl_2$, $MgCl_2$ and NH_4NO_3. An intermediate effect of 1 M NH_4OAc extraction has been marked.

It should be remembered that this does not mean negation or preference of the definite extractors – they reflect some special conditions that may occur in nature. From this reason. they can be appropriate for different applications.

It is not possible to identify one from the analysed set as a universal extractor for estimating available for plants form of all heavy metals.

For different soil conditions and external conditions of extraction can be indicated different preferences for use of specific extractor to estimate the content of bioavailable form of heavy metals.

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MOŻLIWOŚCI OZNACZANIA METALI CIĘŻKICH DOSTĘPNYCH DLA ROŚLIN W GLEBACH TERENÓW PRZEMYSŁOWYCH

Zakład Ochrony i Rekultywacji Gruntów, Instytut Inżynierii Środowiska
Uniwersytet Zielonogórski

Abstract: Do oznaczenia formy biodostępnej metali ciężkich w glebach używane są w gleboznawstwie metodyki oparte o ekstrakcję z użyciem różnych ekstraktorów. Ukazują one analizowaną zawartość w roztworze glebowym oraz w różny sposób związaną z kompleksem sorpcyjnym gleb. Problemem jest wskazanie granicy, poza którą dany pierwiastek można uznać za zabsorbowany trwale. Problem ten jest jeszcze wiekszy w odniesieniu do gleb pod silną antropopresją, których kompleks sorpcyjny podlega wielu działaniom dynamicznie przesuwającym granicę sorpcji i desorpcji. W pracy ukazano wyniki działania na glebę industrialną (Technosols) 1 M roztworami soli: KCl, $MgCl_2$, NH_4NO_3, KNO_3 i NH_4OAc oraz 0.01 M $CaCl_2$, celem wykazania potencjalnej biodostępności Cd, Co, Cu, Ni, Pb i Zn. 1 M roztwory KNO_3 i KCl okazały się relatywnie mocniejszymi ekstraktorami Ni, Cd i Co niż pozostałe. Relatywnie słabymi ekstraktorami wobec analizowanych metali ciężkich są 0.01 M $CaCl_2$, 1 M $MgCl_2$ oraz 1 M NH_4NO_3. Pośrednią mocą odznaczył się 1 M NH_4OAc.

Słowa kluczowe: ekstrakcja metali ciężkich, biodostępne formy metali ciężkich, strefy przemysłowe

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Krystyna HOFFMANN[1*], Marta HUCULAK-MĄCZKA[1]
and Emilia GREK[1]

INVESTIGATION OF THE PROPERTY OF HUMIC ACIDS BY THERMAL ANALYSIS METHOD

BADANIE WŁAŚCIWOŚCI KWASÓW HUMINOWYCH METODĄ ANALIZY TERMICZNEJ

Abstract: The aim of the work was an assessment of the possibilities of using the thermal analysis method in the identification of humic acids. Thermal analysis (TG/DTA) were subjected humic acids extracted from the Belchatów lignite deposit, using the following extraction solutions: 01 M KOH, 0.1 M NaOH and 0.1 M $Na_4P_2O_7$. For comparison, analysis of humic acids purchased from Aldrich company was performed. Thermo-analytical methods determine in examined samples energy change and corresponding changes in mass. It allows to identify the composition of the samples related to the physico-chemical changes that occur during warming. These changes are being recorded on thermograms in the form of endo- and exothermic peaks.

Keywords: humic acids, alkaline extraction, thermal analysis

Introduction

Reserves of the lignite in Poland are counted in amount to 36.9 billion Mg. Main deposits of the lignite are in a Konin Basin, Turow Basin, Belchatow Basin and Sieniawia Lubuska Basin. The lignite is organic, sedimentary combustible rock (caustobiolith) which was formed as a result of the coalifications of plant material, mainly in the period of the Tertiary, exactly of Miocene. The process of the coalification of the plant material takes place in two stages: biochemical (peat, decomposition) and geochemical (diagenesis, metamorphosis). The lignite arises from peat diagenesis in the increased temperature and pressure, and next as a result of the metamorphism is transformed into a hard coal. Table 1 shows an exemplary analytical composition of lignite. Generally the lignite is composed of water, mineral matter and organic substance. In composition of organic matter are included following groups of components: humic acids, fulvic acids, hymatomelanic acids, humins, bitumen, lignin, cellulose. On account of physicochemical properties it is divided on: low coalification

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[1] Institute of Inorganic Technology and Mineral Fertilizers, Wroclaw University of Technology, ul. J. Smoluchowskiego 25, 50–372 Wrocław, Poland, phone: +48 71 320 20 65, email: krystyna.hoffmann@pwr.wroc.pl

lignite (soft), including xsylite and earthy type and high coalification (hard) lignite – matte and shining type [1–3].

Table 1

Analytical composition of the lignite

Moisture	10–70 % mas.
Organic substance Mineral substance	25–89 % 1–25 % mas.

The lower degree of the coalification of the lignite and its calorific value adversely affect on its quality as a fuel (heat of combustion) and its suitability for energy purposes. In the agriculture due to the high content of the humic substance successfully it can be used as a material for production of preparation improving soil quality and also as a component of mineral – organic fertilizers. Humic acids contained in the lignite are included also in the soil organic matter and are characterized by valuable properties of nutrients complexing thus it is appropriate to use them in agriculture, horticulture, arboriculture or gardening [4–7].

Applying mineral fertilizers we provide the necessary for plants development biogenic elements, so-called macroelements – N, P, K, Ca, Mg, S and microelements – Fe, Mn, Zn, Cu, B, Mo and Ni. If in the soil is not enought organic matter, especially macroelements will not be collected by plants and thus it could lead to water eutrophication. This phenomenon depends on the sorption and complexing properties of organic substance. In order to increase use of valuable fertilizer nutrients, while improving the protection of the environment, in the contemporary agriculture more frequently mineral-organic fertilizers instead of mineral fertilizers are used. This action is compatible with the principles of Sustainable Development, one of the acts, which fertilizer industry must obey in the European Union and with various programs and environmental management systems [8–16].

According to the principles of the Sustainable Development the objectives and effects of fertilization in the agriculture should be connected and balanced . From one side it is necessary to provide the food for the still rising number of the world population, on the other side it is sensible to use for this purpose widely understood environment. It should be taken into account, inter alia, utilization of fertilizer raw materials, the damage associated with the production and use of fertilizers as well as utilization of agricultural acreage [17–20].

Low coalification lignite, containing about 60–80 % of humic acids has a greatest importance in the agriculture. To isolate humic acids from the lignite it is subjected to the extraction. Quantity and quality of extracted humic acids depend on the origin of lignite (the kind of flora from which is originated and conditions – temperatures and pressures) and kind of the used extractant. During obtaining humic acids from the lignite occurs series of chemical and physiochemical processes, inter alia, oxidation, nitration, decomposition leading to release gas products (H_2O, CO_2) and depolymerization of macromolecules of the lignite organic substances [6–21].

Humic acids are polymer, amorphous, organic acids, built mainly of carbon, hydrogen and oxygen and small amount of sulphur and nitrogen. The construction basis of humic acids constitute aromatic ring (C_3-C_6) connected bridges, containing functional groups. For specific properties of humic acids which decide about their utilization in the agriculture, maunly include: exchange ability, hydrophilic, acid character and the ability to reversible chemical reactions. It is believed that above properties, humic acids owe their structure, exactly reactive functional groups, mainly – COOH and – OH [6, 9, 11, 13, 22]. Figure 1 shows a molecular structure of humic acids [23].

Table 2 shows the percentage content of major elements included in the humic acids and their potential connections [24].

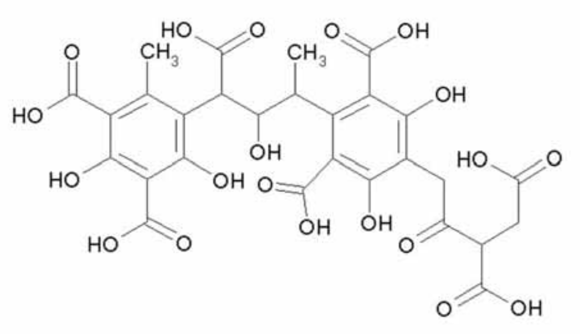

Fig. 1. Molecular structure of humic acids [23]

Table 2

The percentage of major elements included in the humic acids along with the connections they make [24]

Element	Content [% mas.]	Connection
Coal	56–70	– aliphatic chains, – ring-shaped connections – elements of functional groups (-OH, -COOH, >C=O and $–OCH_3$)
Hydrogen	3.3–6.2	
Oxygen	24–33	– heteroatom in the ring – in the form of bridges – elements of functional groups (-OH, -COOH, >C=O and $–OCH_3$)
Nitrogen	3	– heteroatom – amide groups
Sulphur	2	– heteroatom – group $–SO_3H$

Thermal analysis is an analytical technique which allows to determine the thermal effects (DTA) and mass losses (TG) of examined substances. DTG curve is the first derivative of TG curve and reflects the rate of sample mass change with change of temperature. This curve allows a more precise determination of examined value as a temperature function for the points characterizing the changes on the TG curve. These changes occur as a result of physical or chemical transformations and are dependent on the temperature. Method of thermal analysis in combination with mass spectrometry enables the identification of the substance composition. Differential thermal analysis (DTA) uses the energy changes state of the sample influenced by increasing temperature, and the results are visible on the thermogram as endo- and exothermic peaks. Humic substances, after introduction into the soil, undergo many transformations, from which oxidation is most significant. These processes can be analyzed by using the derivatograph [25–30].

Materials and methods

The aim of the study was to evaluate the possibilities of application of thermal analysis to the study of humic acids extracted from Polish lignite deposit. In the research humic acids obtained from Belchatow lignite deposits were used. They were extracted by 0.1M aqueous solution of NaOH, 0.1M aqueous solution of KOH and 0.1M aqueous solution of $Na_4P_2O_7$, where ratio of lignite to aqueous solution was 1:10. Granulation of the lignite was 0.5 mm. Thermal analysis was performed by using derivatograph Netzsch STA 449 F3. Samples were being warmed from temperature 35 ^{o}C to 800 ^{o}C in the small container from Al_2O_3, in the dynamic way, where the temperature increase was 5 ^{o}C/min. Measurements were carried out in air atmosphere, whose flow rate was 30 cm^3/min. Samples were analyzed with mass of about 50 to 90 mg. In order to compare the humic acid extracted from Belchatow lignite deposit, thermal analysis under the same conditions commercial humic acids from Aldrich company were subjected.

Results and discussion

Figure 2 shows the TG and DTA curves for Aldrich humic acids. Similarly, Fig. 3–5 show the TG and DTA curves of humic acids extracted from Belchatow lignite by using 0.1 M NaOH, 0.1 M KOH and 0.1 M $Na_4P_2O_7$.

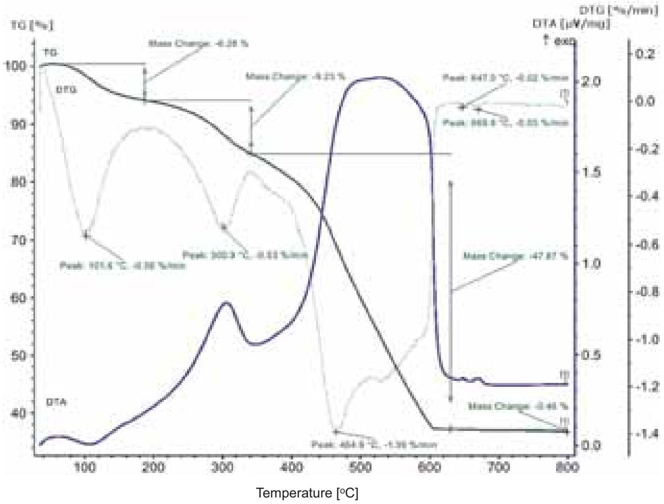

Fig. 2. TG/DTA curves of Aldrich humic acids

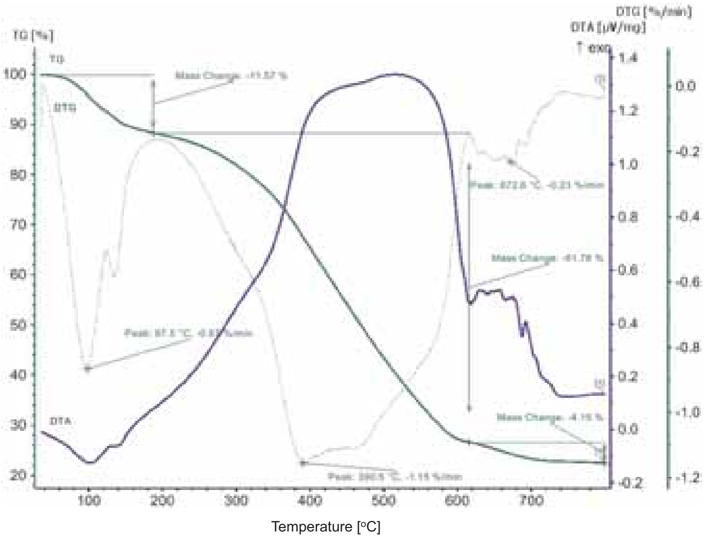

Fig. 3. TG/DTA curves of humic acids extracted from lignite Belchatow with 0.1 M NaOH

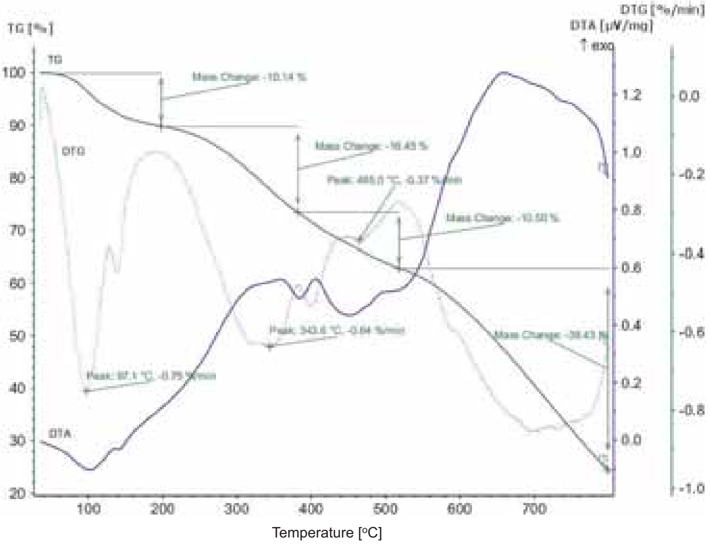

Fig. 4. TG/DTA curves of humic acids extracted from lignite Belchatow with 0.1 M KOH

Thermal analysis of examined samples allows to observe characteristic temperatures of their decomposition, correlated with physical and chemical changes whom are undergone during the measurement. TG and DTG curves enable to estimate mass changes of examined substance during a linear temperature increase. The DTA curve registers the temperature difference between the tested sample and the sample thermally

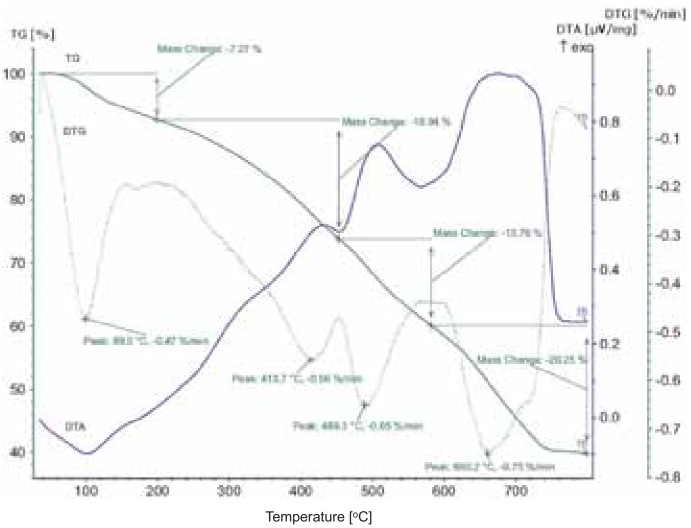

Fig. 5. TG/DTA curves of humic acids extracted from lignite Belchatow with 0.1 M $Na_4P_2O_7$

neutral and in combination with thermogravimetry allows to connect the individual thermal effect with the corresponding transformations, reactions. Table 3 presents the collected results of analysis.

Table 3

The results of thermal analysis (effects of endo- and exothermic) Aldrich humic acids and humic acids extracted from lignite from Belchatow deposit

Sample	Maximum temperature of the disintegration [°C]	Temperature range [°C]	Weight loss [% mas.]	Residue after the process [% mas.]
KH from Aldrich company	101.6	40–180	6.28	36.36
	300.9	180–340	9.23	
	464.9	340–625	47.67	
	669.8	625–800	0.46	
KH Bel. – KOH	97.1	40–200	10.14	24.48
	343.6	200–380	16.45	
	465.0	380–520	10.50	
	620–780	520–800	38.43	
KH Bel. – NaOH	97.5	40–180	11.57	22.50
	390.5	180–605	61.78	
	672.6	605–800	4.15	
KH Bel. – $Na_4P_2O_7$	99.0	40–200	7.27	39.81
	413.7	200–450	18.94	
	489.3	450–580	13.76	
	660.2	580–800	20.25	

The first, endothermic peak occurs both in a sample of humic acids commercial (Aldrich) and those obtained from lignite. This peak occurs near 100 ^{o}C and is associated with moisture loss (dehydration). A small shifts of the peaks due to the fact that water is characterized by varying a different degrees of binding. Above this temperature, followed by degradation of organic matter, which is accompanied by exothermic effect. Transformations between 200–400 ^{o}C are most likely the result of combustion of polysaccharides, degradation of functional groups and phenolic compounds. This conversion is clearly visible on the graph of commercial humic acid (Aldrich). However on the graphs of tested humic acids, obtained in laboratory conditions from lignite using alkaline extraction method significant change are not observed. The loss of mass in this temperature range varies from 9.23 % for commercial humic acids to 61.78 % for humic acids extracted with NaOH. Humic acids extracted with KOH and $Na_4P_2O_7$ have a similar value of the mass loss for temperature range (about 20 %).

The next exothermic peaks is visible in the range of temperature 400–600 ^{o}C and it is associated with the burning of aromatic structures (alkyl aromatic compounds), of polyphenols, decomposition of C-C bonds. In case of Aldrich humic acids, this temperature corresponds the largest weight loss – 47.67 %. That means that the acids contain most of all thermally stable compounds. Humic acids extracted with KOH and $Na_4P_2O_7$ have again a similar value of the mass loss for this range of temperatures – about 10 %. In case of humic acids extracted with NaOH there is a lack of clear boundaries between these ranges of temperatures. On the shown thermogram there is only one large exothermic peak in the temperature range between 180–605 ^{o}C, accompanied by 61.78 % weight loss. This effect is not observed in the case of humic acids purchased from Aldrich company.

The last exothermic effect occurs at the temperatures above 600 ^{o}C and it is probably associated with high-temperature policondensation reactions, depolymerization, pyrolysis. This effect is mainly observed for humic acids extracted in laboratory conditions. The thermogram in Fig. 4 shows that the process at 800 ^{o}C has not yet ended, moreover, precisely in this temperature range 550–650 ^{o}C there is the greatest loss of weight.

Summary

Thermal analysis methods allow to obtain quantitative and qualitative data of the investigated substances. DTA method is useful in the investigation of structural changes occurring during heating of the sample. TG and the first derivative of TG-DTG allow to quantitatively characterize the mass loss of substances. The analysis of obtained derivatograms show that the composition of humic acids obtained in the laboratory condition by alkaline extraction method is variable and depends on the used extractant – extracted humic acids have variable chemical composition and structure.

In conclusion, the use of thermal analysis to the physicochemical characteristics of humic acids is useful and can provide a basis for further research on the possibilities of its application.

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BADANIE WŁAŚCIWOŚCI KWASÓW HUMINOWYCH METODĄ ANALIZY TERMICZNEJ

Instytut Technologii Nieorganicznej i Nawozów Mineralnych
Politechnika Wrocławska

Abstrakt: Celem pracy była ocena możliwości wykorzystania metody analizy termicznej w identyfikacji kwasów huminowych. Analizie termicznej (TG/DTA) poddano kwasy huminowe ekstrahowane z bełchatowskiego złoża węgla brunatnego, przy użyciu 0,1 M KOH, 0,1 M NaOH i 0,1 M $Na_4P_2O_7$. Dla porównania wykonano analizę odczynnikowych kwasów huminowych firmy Aldrich. Metody termoanalityczne określają zamiany energetyczne i odpowiadające im zmiany masowe. Pozwala to na identyfikację składu badanych próbek związanych z przemianami fizykochemicznymi zachodzącymi podczas ogrzewania. Zmiany te rejestrowane są na termogramach w postaci pików endo- i egzotermicznych.

Słowa kluczowe: kwasy huminowe, ekstrakcja alkaliczna, analiza termiczna

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Ewa ŁOBOS-MOYSA[1]

EVALUATION OF CONTAMINATION LEVEL OF GLIWICE CHANNEL AND ITS SIDE STREAMS WITH ORGANIC COMPOUNDS

OCENA STOPNIA ZANIECZYSZCZENIA ZWIĄZKAMI ORGANICZNYMI KANAŁU GLIWICKIEGO I JEGO WYBRANYCH DOPŁYWÓW

Abstract: The aim of the study was to determine the level of contamination of different side streams of Gliwice Channel basing on analyses of organic compounds concentrations. Surface waters localized near two Silesian cities *ie* Zabrze and Gliwice were analyzed during the study *ie* Mao Stream (Wojtowianka) (1 sampling point), Rokitnica Stream (1 sampling point), Bytomka (1 sampling point), Klodnica River (2 sampling points) and Gliwice Channel (1 sampling point). Water samples were collected twice a month in the winter-spring-summer period, always in the morning. COD analysis was made via dichromate method using NOVA 400 spectrophotometer by Merck. BOD_5 was determined via respirometric method with Oxi Top equipment by WTW and TOC by multi N/C Analytik Jena. Physico-chemical parameters of investigated waters *ie* pH and temperature were measured using Elmetron set equipped with proper electrodes. Obtained results were elaborated according to physico-chemical parameters of investigated waters (pH, suspended solid concentration) and watercourse properties (length, number of side streams, flowing area characteristic).

Keywords: natural watercourses, organic compounds, BOD_5, COD, TOC

Introduction

Surface waters present at highly industrialized and urban areas like Upper Silesia become seriously contaminated by humans activities. The main sources of contaminants are municipal and industrial wastewaters together with water flows from contaminated areas including industrial ones [1, 2]. One of the most important industry branch which has a great impact on environment is mining [3, 4]. The exemplary river damaged by this activity is Klodnica River, which has been highly contaminated for almost 100 years by now [5].

The study focused on determination of contamination level (basing on organic compounds concentrations measurements) of various water courses which are main side

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[1] Faculty of Energy and Environmental Engineering, Silesian University of Technology, ul. Akademicka 2A, 44–100 Gliwice, Poland, phone: +48 32 237 29 81, email: ewa.lobos-moysa@polsl.pl

streams of Gliwice Channel. As most of the investigated water courses are found to be contaminated or highly contaminated basic wastewater parameters i.e. BOD_5, COD and TOC were used for their evaluation [5, 6]. Oxidability was not date.

Methodology of the study

Following streams, rivers and artificial channel localized at Gliwice and Zabrze cities areas were used for the study *ie* Doa Stream (Wojtowianka) (1 sampling point N50°16′45″ E18°39′0″), Rokitnicki Stream (1 sampling point N50°21′54″ E18°48′8″), Bytomka River (1 sampling point N50°18′32″ E18°46′41″), Klodnica River (2 sampling points: N50°18′37″ E18°39′0″ and weir) and Gliwice Channel (1 sampling point N50°20′25″ E18°37′12″). Doa and Rokitnicki Streams, through Ostropka and Bytomka Rivers, are Klodnica River side streams, while Klodnica River is one of the side streams of Gliwice Channel. Water samples were collected twice a month in 2010 during winter-spring-summer period (in the sampling day morning). All samples were collected manually to dark-glass bottles. The quality control of water can be made via analysis of physical parameters and chemical parameters *eg* BOD_5, COD, TOC, pH, suspended solids [7]. BOD_5 analysis was made via manometric method using Oxi Top system by WTW. Total and suspension free (*ie* filtered samples) COD was determined by means of dichromate method on NOVA 400 spectrometer by Merck. Total and suspension free TOC was analyzed using TN-TC multi N/C analyzer by Analytik Jena. Physical parameters of samples *ie* temperature and pH were measured with the use of Elmetron set equipped with proper electrodes. Dry mass content in the suspension was determined at 105 °C by weight method.

Results and discussion

The indicators of water pollution

Physico-chemical analyses of collected surface water samples revealed that the degree of contamination of investigated waters with organic compounds (BOD_5, COD and TOC) and suspended solids was different despite all water courses were localized at Upper Silesia (Fig. 1–5). Doa and Rokitnicki Streams characterized with lowest parameters values in the range of: for BOD_5 – up to 5 mgO_2/dm^3 and up to 10 mgO_2/dm^3, for COD from 26.5 to 52 mgO_2/dm^3 and from 24 to 58 mgO_2/dm^3, for TOC from 9 to 10 mg/dm^3 and from 7 to 11 mg/dm^3, respectively. Bytomka and Klodnica River together with Gliwice Channel were defined as contaminated surface waters [8]. The measured BOD_5 values of those water courses were almost at the same level and in the range of 10 mgO_2/dm^3 to 30 mgO_2/dm^3, 10 mgO_2/dm^3 to 40 mgO_2/dm^3 and 5 mgO_2/dm^3 to 35 mgO_2/dm^3, respectively. The greatest impact of anthropogenic activity on investigates surface waters activity was observed at COD parameter. It value was in the range of 48 mgO_2/dm^3 to 98 mgO_2/dm^3, 52 mgO_2/dm^3 to 174 mgO_2/dm^3 and 51 mgO_2/dm^3 to 145 mgO_2/dm^3 for Bytomka River, Klodnica River and Gliwice Channel respectively. The contamination of investigated waters with organic compounds was accompanied by high suspended solids content (Fig. 4). However,

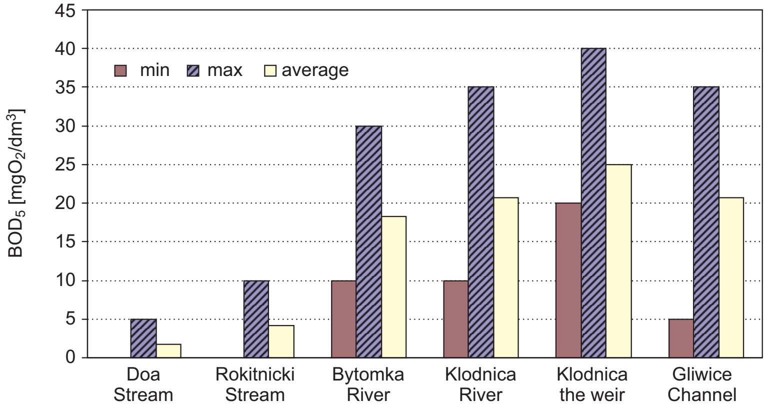

Fig. 1. BOD_5 of investigated waters

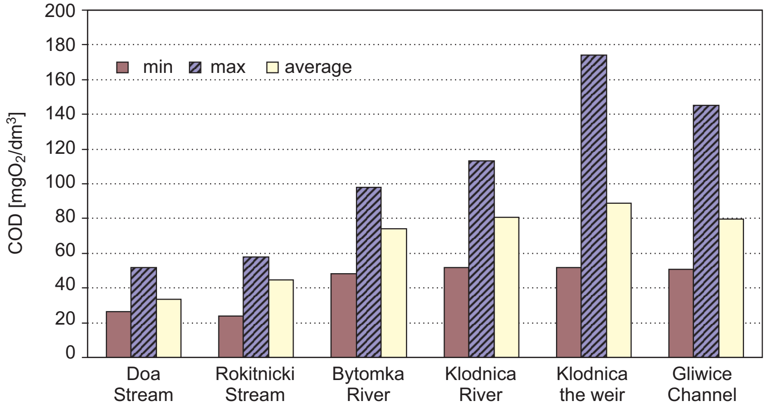

Fig. 2. Total COD of investigated waters

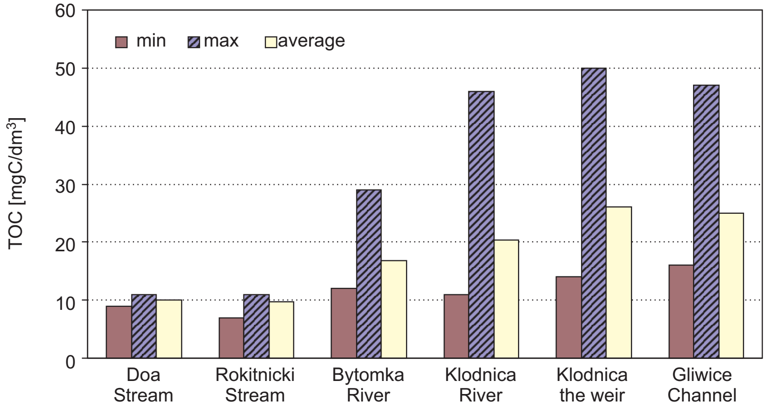

Fig. 3. TOC of investigated waters

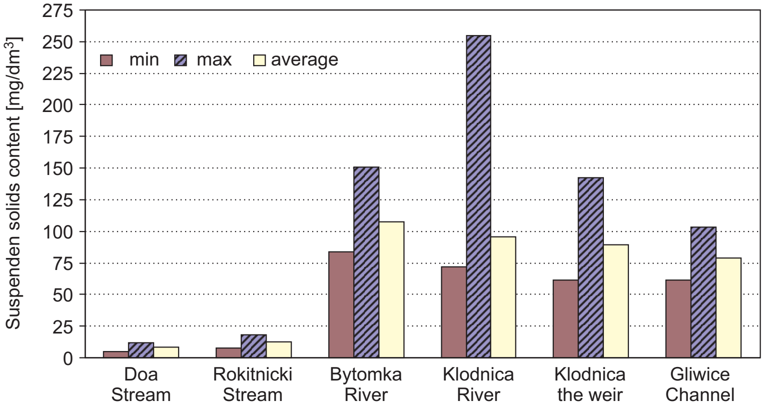

Fig. 4. Suspension of investigated waters

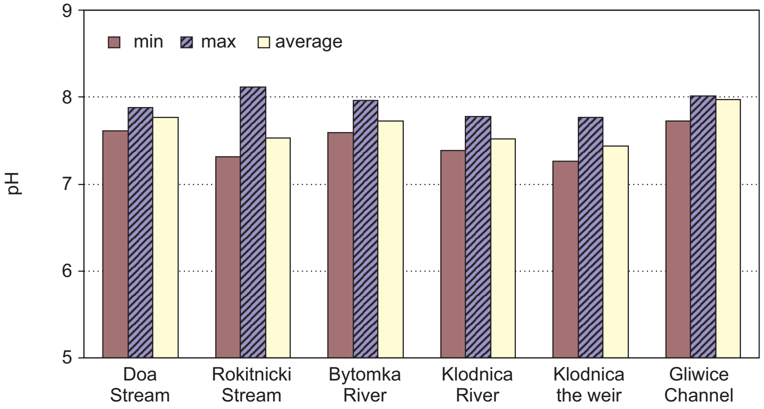

Fig. 5. pH of investigated waters

measured valued of pH parameter did not indicate on such a high contamination as they were in the range of 7.26 to 8.12 (Fig. 5).

Mean values of water contamination parameters with organic compounds are presented in Fig. 6. Doa Stream (periodically dry stream of length 4 km) was found to be the least contaminated water course. It flows through agricultural areas and has an estuary in Ostropka, which is Klodnica River side stream. Similarly low parameters values were obtained for Rokitnicki Stream for which the sampling place was localized at low population density area (between two districts of Zabrze *ie* Helenka and Rokitnica). Moreover, up to the sampling place Rokitnicki Stream flows mainly through fields. However, after passing Zabrze city center at the estuary to Bytomka River (Gliwice city area) Rokitnicki Stream becomes highly contaminated surface water.

In case of other investigated waters sampling places were localized at high population density areas. Besides, before reaching the sampling point those water courses flow through urban and industrial areas. The sampling place for Bytomka River

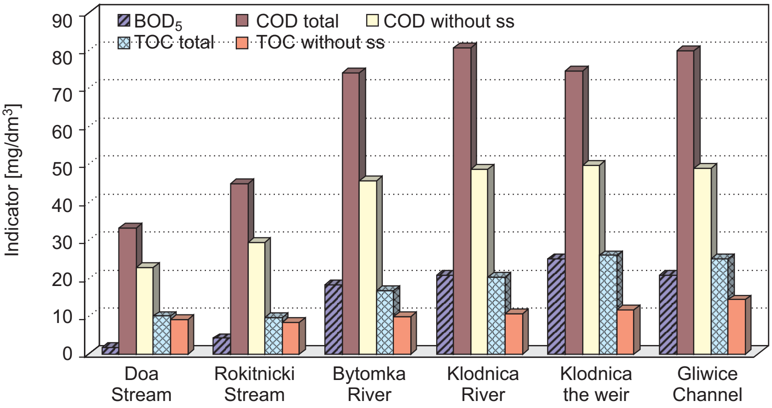

Fig. 6. Average values of indicators of water pollution

was localized at Zabrze city center after its flow through Bytom and Ruda Slaska. In case of Klodnica River, two sampling places were chosen *ie* before Central Municipal Wastewater Treatment Plant and at the weir after the Plant at which it already flowed through Katowice, Ruda Slaska, Zabrze and Gliwice. Samples of Bytomka and Klodnica Rivers and Gliwice Channel waters indicated on their high contamination considering both, chemical (BOD_5) and industrial (COD-Cr) parameters of water quality (Fig. 6). According to the literature the side streams of Klodnica River have the great anthropogenic impact on its water quality [6, 9, 10]. It was found that side streams of Klodnica River *ie* contaminated Bytomka and Kochlowka had an unfavorable impact on its BOD_5 parameter value, while its COD was mainly affected by Bytomka, Bielszowicki Stream and Czarniawka waters incomes [6, 11]. Similar dependences were obtained in case of total suspended solid content analysis [5]. Thus, Klodnica River has negative impacts on other surface waters [12].

Nowadays anthropogenic pollutants as the indicators of water pollution

The recent papers show the improvement of Polish surface water quality in refer to one observed in seventies of XX century caused by both, the collapse of large part of industrial plants but also by the increase of ecological consciousness [12, 13]. Nevertheless, the detailed analysis of surface water quality establishing the level of organic macro- and micropollutants is needed. On the one hand the concentration of macropollutants is quite easy to be monitored and their load to the environment is limited by regulations on wastewater treatment. On the other hand, the concentration micropollutants still increases [14]. The development in chemical analysis enables the simultaneous monitoring of wide range of compounds in aqueous samples [2, 15]. In the previous century the main groups of compounds were PAHs, surfactants, pesticides and chlorinated organic compounds [16, 17]. Nowadays among anthropogenic pollutants pharmaceuticals are of the highest interest, while among natural ones mycoestrogens [14, 18, 19].

The characteristic of surface waters based on BOD_5, COD and TOC parameters values enabled to determine the degree of their contamination. Those are general water parameters thus their evaluation allows only to conclude, that water contamination is caused by humans activities [20]. In order to determine exact contamination sources more detail analyses of number of chemical compounds must be performed. The main indicator of water contamination with untreated municipal wastewater is the presence of the long-chain fatty acids (as components of eatable oils and fats). In case of water contamination with industrial wastewaters characteristic organic compounds *eg* phenols are said to be so-called industrial indicators [6].

Moreover it can be show that the water pollution is caused by municipal wastewater deposition as biological indicators *ie Streptococcus Faecalis* or *Clostridi*a resting spores [21] can be determined.

The total content of the three long-chain fatty acids for surface water as the proposed indicators at highly industrialized and urban areas shown in Fig. 7. The relationship

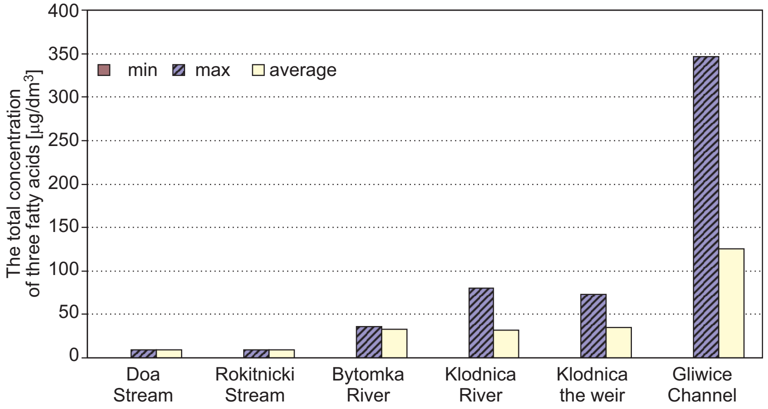

Fig. 7. The total content of the three long-chain fatty acids for surface waters

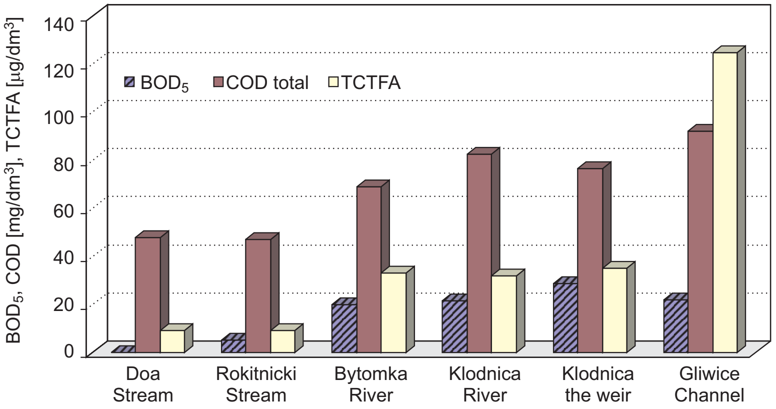

Fig. 8. Average values of BOD_5, COD and total content of the three long-chain fatty acids for surface waters

between indicators is given in Fig. 8. Natural waters which characterized with high BOD_5 and COD values revealed also the highest concentration of fatty acids concentration of which was the most unstable.

Conclusion

1. The study of various water courses which are main side streams of Gliwice Channel showed the influence of the human activity on the natural environment.
2. Doa Stream flows through agricultural areas as Ostropka River side streams. It characterized with lowest organic pollutants (BOD_5, COD). Similarly, low parameters values were obtained for Rokitnicki Stream for which the sampling place was localized at low population density area.
3. Rokitnicki Stream (after passing Zabrze city center), Bytomka River and Klodnica River became highly contaminated surface waters. The influence of the anthropogenic pollutants was confirmed both using COD as well as long-chain fatty acids. The water quality of side streams influences on the water quality of the Gliwice Channel.
4. The characteristic of surface waters based on BOD_5, COD and TOC parameters values enabled to determine the degree of their contamination. In order to determine exact contamination sources more detail analyses of number of chemical compounds must be performed. Determination of the nowadays anthropogenic organic pollutants is very important and necessary. The main indicator of water contamination with untreated municipal wastewater or wastewater from food industry can be the presence of the long-chain fatty acids

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OCENA STOPNIA ZANIECZYSZCZENIA ZWIĄZKAMI ORGANICZNYMI KANAŁU GLIWICKIEGO I JEGO WYBRANYCH DOPŁYWÓW

Wydział Inżynierii Środowiska i Energetyki
Politechnika Śląska

Abstrakt: Badania miały na celu określenie stopnia zanieczyszczenia różnych cieków wodnych zasilających Kanał Gliwicki na podstawie oznaczenia związków organicznych. Do badań wybrano wody powierzchniowe znajdujące się na terenie Gliwic i Zabrza: Doa (Wójtowiankę), Potok Rokitnicki, Bytomkę, Kłodnicę oraz Kanał Gliwicki. Próby pobierano dwa razy w miesiącu w okresie od zimowo-wiosenno-letnim w godzinach porannych w ciągu jednego dnia. Oznaczenie ChZT wykonano metodą dwuchromianową przy użyciu spektrofotometru NOVA 400 Merck; BZT_5 metodą manometryczną, przy użyciu buteleczek Oxi Top firmy WTW, OWO na analizatorze TN-TC multi N/C firmy Analytik Jena. Suchą masę zawiesiny oznaczano metodą wagową. Uzyskane wyniki opracowano w zależności od parametrów fizykochemicznych badanych cieków (pH, stężenia zawiesiny) oraz ich charakterystyki (długości, ilości dopływów, charakteru terenów przez które płyną).

Słowa kluczowe: naturalne cieki, zanieczyszczenia organiczne, ChZT, BZT_5, OWO

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Sebastian WERLE[1]

SEWAGE SLUDGE AS AN ENVIRONMENTAL FRIENDLY ENERGY SOURCE

OSADY ŚCIEKOWE ŹRÓDŁEM ENERGII PRZYJAZNEJ ŚRODOWISKU

Abstract: The predominant method of the sewage sludge management in Poland is land disposal. However, since 01/01/2016, this method will be prohibited. Therefore, there is a strong need for development of thermal methods of sludge disposal. In the Polish legal system sewage sludge may be named as a biomass or waste. For purposes of determining the obligations of environmental regulations definition of the Minister of Environment should be used. When disposing of sewage sludge in an amount up to 1 % by weight of fuel, emission standards for fuel do not change. At the disposal of sewage in quantities of more than 1 %, should be conducted continuous measurement of emissions, including HCl, HF, and continuous measurements of flue gas parameters (as for the installation of waste disposal). For purposes of settlement of the share of energy from renewable sources we use the definition of Minister of Economy. In this case, in accordance with applicable law sewage sludge shall be considered as pure biomass is CO_2 neutral. The use of sewage sludge as a fuel requires the determination of fundamental combustible properties. These properties should be in accordance with the requirements put fuels as an energy source. The paper presents results of a detailed physico-chemical analysis of dried sewage sludge produced in the two Polish wastewater treatment plants. The results were compared with five representatives of biomass fuels: straw of wheat, straw of rape, willow, pine and oak sawdust. Ultimate and proximate analysis includes a detailed analysis of fuel and ash. The results clearly indicate that the sludge is a very valuable fuel similar to "traditional" biomass.

Keywords: sewage sludge, thermal treatment, combustible properties

Introduction

Sewage sludge, originating from the treatment process of wastewater, is the residue generated during the primary (physical and/or chemical), the secondary (biological) and the tertiary (additional to secondary, often nutrient removal) treatment [1–3]. Removal of sludges from *Wastewater Treatment Plants* (WWTP) represents a serious worldwide environmental problem. Not long ago, it was thought that raw sludge was a valueless material that should be discarded, and then it was disposed of in landfills and/or thrown into the ocean. But the huge amounts of sludge produced make all these options

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[1] Institute of Thermal Technology, Silesian University of Technology, ul. Konarskiego 22, 44–100 Gliwice, Poland, phone: +48 32 237 29 83, email: sebastian.werle@polsl.pl

environmentally unacceptable. The high output of sewage sludge, which is increasing during recent years, and the limitations of the existing means of disposing sewage sludge highlight the need to find alternative routes to manage this organic material. The *6th Environment Action Programme 2002–2012* of the European Commission has been described as a major factor in reducing sewage sludge disposal by 50 % from 2000 by 2050. Moreover, European legislation prohibits the deposition of sewage sludge into landfill or water. Biomass and residues like sewage sludge are the only renewable energy sources that can provide C and H, thus it is interesting to process them by means of treatments that enable to obtain chemically valuable products like fuels. As a type of biomass fuel, sewage sludge is a renewable source and has advantage of being CO_2-neutral: no additional CO_2 is estimated into the atmosphere in the long term. The latest trends in the field of biomass and sludge management, (*ie*, combustion, pyrolysis, gasification and co-combustion) have generated significant scientific interest [4]. Those processes are illustrated in the flow-chart shown in Fig. 1.

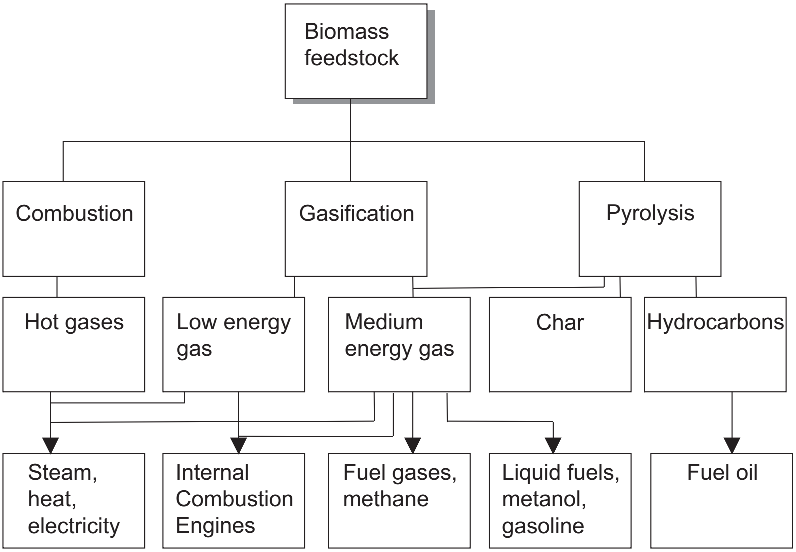

Fig. 1. Main processes of biomass conversion

Gasification is the process of converting a solid fuel into a gas by treating the solid fuel in a generator with oxygen, air, and steam or by other gasification methods [5]. The most important reactions that take place in the reduction zone of a gasifier between the different gaseous and solid reactants are given below. A minus sign indicates that heat is generated in the reaction, a positive sign that the reaction requires heat.

$$CO + CO_2 \rightarrow 2CO_2 + 164.9 \text{ kJ/kmol} \tag{1}$$

$$C + H_2 \rightarrow CO + H_2 + 122.6 \text{ kJ/kmol} \tag{2}$$

$$CO + H_2 \rightarrow CO + H_2O + 42.3 \text{ kJ/kmol} \tag{3}$$

$$C + 2H_2 \rightarrow CH_4 + 0 \text{ kJ/kmol} \tag{4}$$

$$CO + 3H_2 \rightarrow CH_4 + H_2O - 205.9 \text{ kJ/kmol} \tag{5}$$

As shown in Marrero et al, gasification of sewage sludge leads to a high-quality flammable gas that can be used for the generation of electricity or to support such processes as the drying of sewage sludge [6]. Gasification is one way of using sewage sludge and is an attractive alternative to other treatment methods. To determine the usefulness of sewage sludge as a biomass fuel for thermal transformation, it is necessary to know its basic physical and chemical characteristics. The elemental composition of sewage sludge and the contents of inorganic compounds depend on many factors, but it may be largely dependent on the country or region of origin.

The aim of the work is comparison of physico-chemical properties of dried sewage sludge produced in the two Polish wastewater treatment plants with five representatives of "traditional" biomass fuels: straw of wheat, straw of rape, willow, pine and oak sawdust. Ultimate and proximate analysis includes a detailed analysis of fuel and ash.

Results

Within this study straw of wheat and rape, oak, willow and pine sawdust and two sewage sludge samples were examined. The proximate and ultimate analysis are presented on Fig. 2.

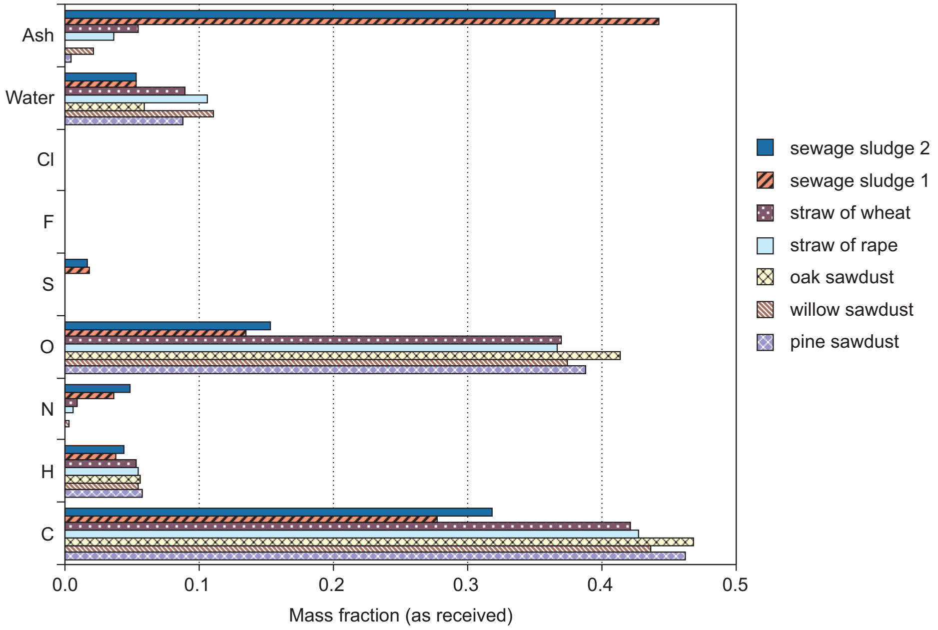

Fig. 2. Ultimate analysis and ash and water content in analysed feedstock

The moisture of the feedstock was obtained following standard PN-EN 14774-3:2010 [7]. The infrared spectroscopy analyzer was used to carry out the ultimate analysis of the sewage sludge.

The volatile matter content was determined according to standard PN-EN 15402:2011 [8]. The ash content was obtained using PN-EN 15403:2011 [9]. The calorific content was determined in accordance with standards CEN/TS15400:2006 [10].

As it can be seen from ultimate analysis, there are no significant differences in the C, H, Cl and F content. Nevertheless, taking into consideration S, N and O contents those difference between "traditional" biomass and sewage sludge is quite strong. Despite the fact that sewage sludge contains phosphorus, nitrogen and sulfur, the gasification of these components offers several advantages over a traditional combustion process. Gasification takes place in an environment with low levels of oxidizers (to prevent the formation of dioxins) and large quantities of sulfur and nitrogen oxides [11]. As mentioned above, sulfur is present in sewage sludge at low amounts; it is mainly converted to hydrogen sulfide (H_2S) during gasification [12], whereas the nitrogen is transformed into ammonia [11].

It is worth noting that the phosphorus in sewage sludge is partitioned into solid (not gaseous) residues [13] and that the volume of syngas produced from sewage sludge is low because gasification requires a fraction of the stoichiometric amount of oxygen necessary for combustion. For all of these reasons, gasification requires smaller and less expensive gas-cleaning facilities [14]. Analysing Fig. 2 it can be also seen that the sewage sludges were characterized by higher ash content than "traditional" biomass feedstock.

It can be seen in Fig. 3 that lower heating value is comparable to that of traditional biomass. Simultaneously in Fig. 4, it can be observed that volatile matter content in the sewage sludge is much lower in comparison with traditional biomass. The combination of low oxygen content and low volatile matter in sewage sludge indicates a low potential for creating large amounts of inorganic vapors during combustion and another thermal processes.

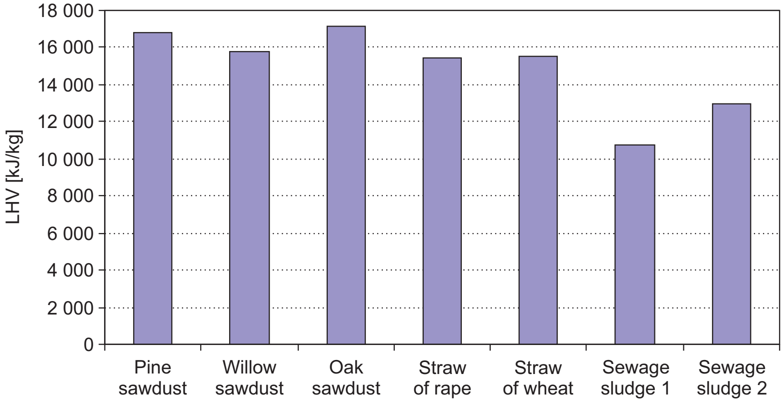

Fig. 3. Lower heating value of analysed feedstock

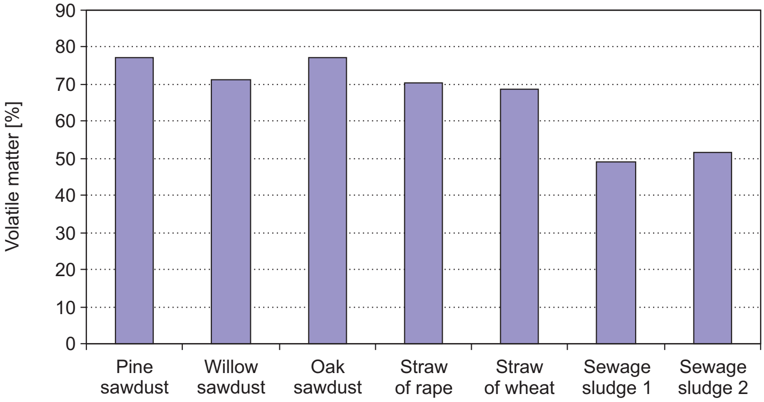

Fig. 4. Volatile matter content of analysed feedstock

The results of the chemical analyses of the fly ashes are presented in Fig. 5. The plasma spectrometer Thermo iCAP 6500 Duo ICP was used to carry out the ash analysis of the biomass feedstock.

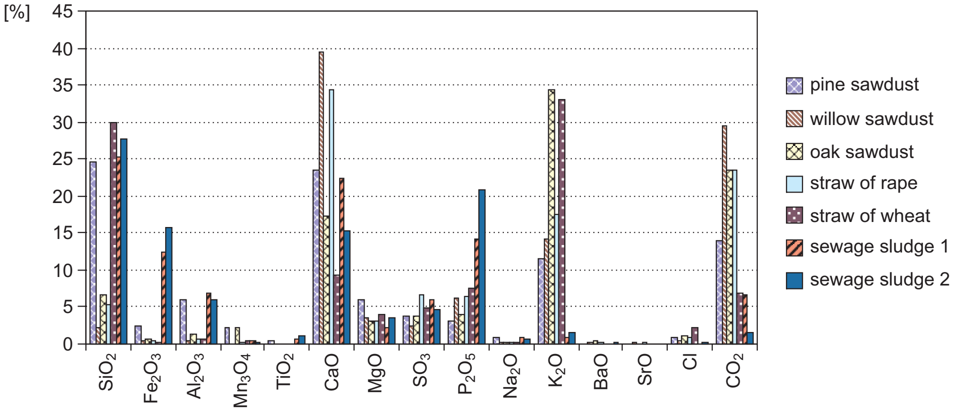

Fig. 5. Ash chemical analysis of analysed feedstock

Ash behavior and deposition tendencies were predicted through the use of empirical indices for biomass type ashes [15–17]. These indices, despite their shortcomings due to the complex conditions, which arise in boilers and their associated heat transfer equipment, are widely used and probably remain the most secure basis for decision making, if used in conjunction with pilot plant testing.

One simple index, the *alkali index* (AI) which is a parameter frequently used to describe the overall influence of catalytically active species within the ash and is defined as the ratio of the sum of the fraction of the basic compounds in the ash (CaO, MgO, K_2O, Na_2O and Fe_2O_3) to the fraction of the acidic compounds (SiO_2 and Al_2O_3) in the ash, multiplied by the ash value (eq. (1)).

$$AI = ash\% \cdot \frac{CaO + MgO + K_2O + Na_2O + Fe_2O_3}{SiO_2 + Al_2O_3} \tag{1}$$

When the AI increases slagging tendency increases.

Another index, the *base-to-acid ratio* (B/A) – eq. (2). As B/A increases, the fouling tendency of a fuel ash increases.

$$B/A = \frac{\%(CaO + MgO + K_2O + Na_2O + Fe_2O_3)}{\%(SiO_2 + TiO_2 + Al_2O_3)} \tag{2}$$

A *bed agglomeration index* (BAI) – eq. (3) has been developed, relating ash composition with agglomerations in fluidized bed reactors. BAI was calculated according to the following equation.

$$BAI = \frac{\%Fe_2O_3}{\%(K_2O + Na_2O)} \tag{3}$$

Bed agglomeration occurs when BAI values become lower than 0.15.

Analysing data presented in Fig. 6 it can be concluded that sewage sludge is characterizing by higher slagging tendency in comparison with traditional biomass (especially oak and pine sawdust). Simultaneously sewage sludge ash is characterised by lower fouling tendency than traditional biomass ash and higher tendency to create agglomerates.

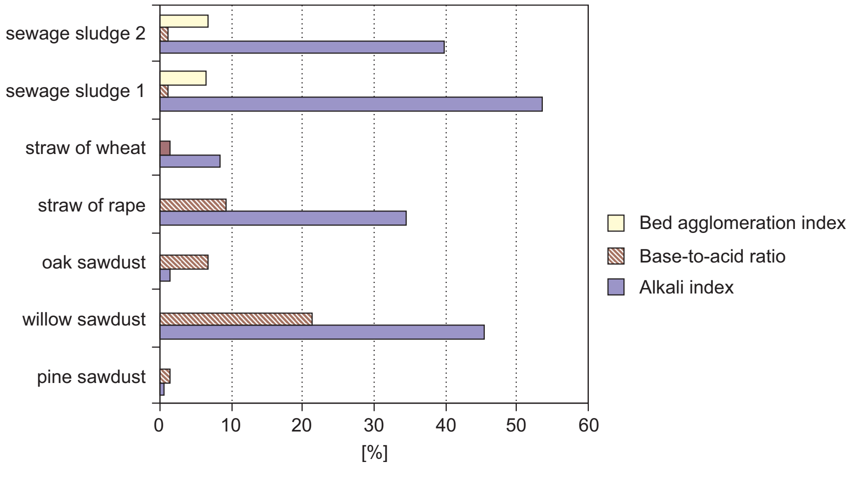

Fig. 6. Slagging/fouling indexes of analysed feedstock

Figure 7 shows the content of trace elements measured in the ashes of the studied fuels and – additionally to compare – hard coal and biomass ashes. The measurements was done by absorption spectroscopy. The measured values in all presented cases are

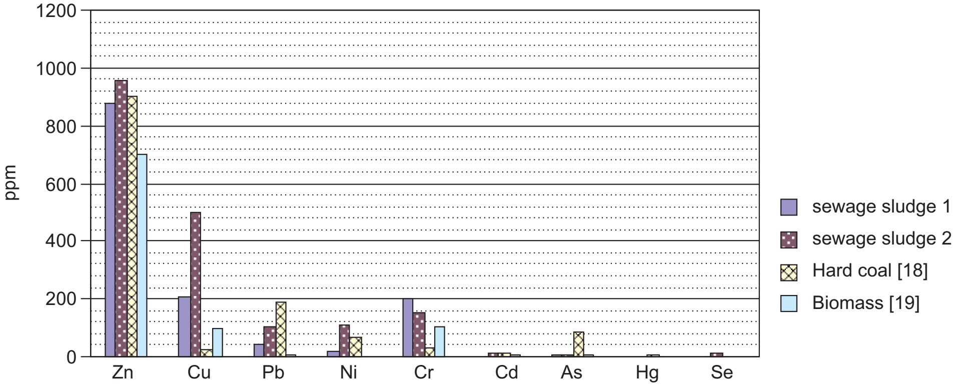

Fig. 7. Trace and selected minor elements measured in ashes

similar. It should be emphasis that sewage sludge gasification ash taking in consideration trace elements is similar to traditional biomass.

Conclusions

The analysis of various biomass materials indented to be used as supplemental fuel in fossil fuel fired power plants has shown that there is always a range of the results sometimes with a big gap between minimum and maximum. Most noticeable for the sewage sludge was the highest share of ash, nearly 50 % of the dry substance, compared with all the other fuels. Additionally it should be emphasis that the combination of low oxygen content and low volatile matter in sewage sludge indicates a low potential for creating large amounts of inorganic vapors during combustion and another thermal processes. Moreover, sewage sludge is characterizing by higher slagging tendency, lower fouling tendency and higher tendency to create agglomerates in comparison with traditional biomass.

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OSADY ŚCIEKOWE ŹRÓDŁEM ENERGII PRZYJAZNEJ ŚRODOWISKU

Instytut Techniki Cieplnej
Politechnika Śląska w Gliwicach

Abstrakt: Dominującym kierunkiem zagospodarowania osadów ściekowych w Polsce jest ich składowanie. Jednakże począwszy od 1.01.2013 sposób ten będzie zabroniony. Istnieje zatem silna potrzeba rozwoju termicznych metod utylizacji osadów. W polskim ustawodawstwie osad może być nazywany biomasą lub odpadem. Dla celów ustalenia, jakie obowiązki wynikają z przepisów ochrony środowiska, korzystać należy z definicji Ministra Środowiska. Przy utylizacji osadów ściekowych w ilości do 1 % masy paliwa, standardy emisyjne dla paliw nie ulegają zmianie. Przy utylizacji osadów w ilości ponad 1 %, należy prowadzić ciągły pomiar emisji zanieczyszczeń, w tym HCl i HF, a także ciągły pomiar parametrów spalin (jak dla instalacji utylizacji odpadów). Do potrzeb rozliczenia udziału energii pochodzącej ze źródeł odnawialnych stosuje się definicję Ministra Gospodarki. W takim przypadku, zgodnie z obowiązującym prawem osady ściekowe uznaje się za czystą biomasę neutralną pod względem CO_2. Wykorzystanie osadów ściekowych jako paliwa wymaga określenia podstawowych właściwości palnych. Właściwości te powinny odpowiadać wymaganiom, jakie są stawiane paliwom w celu ich energetycznego wykorzystania. W pracy przedstawiono wyniki szczegółowej analizy fizykochemicznej suszonych osadów ściekowych wytworzonych w dwóch polskich oczyszczalniach ścieków. Wyniki zostały porównane z pięcioma przedstawicielami paliw biomasowych: słomy pszennej, rzepakowej, wierzby energetycznej, trocin sosnowych i dębowych. Analiza obejmowała skład elementarny paliw oraz szczegółową analizę popiołu. Wyniki jednoznacznie wskazują, iż osady ściekowe są bardzo wartościowym paliwem nieróżniącym się w zasadniczy sposób od „klasycznej" biomasy.

Słowa kluczowe: osady ściekowe, termiczna utylizacja, własności palne

Varia

INVITATION FOR
ECOpole ’13 CONFERENCE

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 22nd annual Central European Conference ECOpole ’13, which will be held in 23–26.08.2013 (Wednesday–Saturday) in Hotel Ziemowit in Jarnoltowek, PL.

The Conference Programme includes oral presentations and posters and will be divided into five sections:

- **SI Chemical Pollution of Natural Environment and Its Monitoring**
- **SII Environment Friendly Production and Use of Energy**
- **SIII Forum of Young Scientists and Environmental Education in Chemistry**
- **SIV Impact of Environment Pollution on Food and Human Health**

The Conference language is English.

Contributions to the Conference will be published as:

- abstracts on the CD-ROM (0.5 page of A4 paper sheet format),
- extended Abstracts (5–8 pages) in the semi-annual journal *Proceedings of ECOpole*,
- full papers will be published in successive issues of the *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (Ecol. Chem. Eng.) ser. A or S.

The deadline for sending the Abstracts is **15th July 2013** and for the Extended Abstracts: **1st October 2013**. The actualized list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one can find (starting from **31st July 2013**) on the Conference website:

ecopole.uni.opole.pl

The papers must be prepared according to the Guide for Authors on Submission of Manuscripts to the Journals.

The Conference fee is 400 € (covering hotel, meals and transportation during the Conference). It could be reduced (to 250 €) for young people actively participating in the Forum of Young Scientists. But the colleague has to deliver earlier the Extended Abstract (5–8 pages) of his/her contribution (deadline is on **15.08.2013**), and a recommendation of his/her Professor.

Fees transferred after **1st September 2013** are 10% higher.

At the Reception Desk each participant will obtain abstracts of the Conference contributions as well as the Conference Programme recorded on electronic media (the Programme will be also published on the ECOpole ’13 website).

After the ECOpole ’13 Conference it will be possible to publish electronic version of presented contributions (oral presentations as well as posters) on the website.

Further information is available from:
Prof. dr hab. inż. Maria Wacławek
Chairperson of the Organising Committee
of ECOpole ’13 Conference
University of Opole
email: Maria.Waclawek@o2.pl
and mrajfur@o2.pl
phone: +48 77 401 60 42
fax +48 77 401 60 51

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ZAPRASZAMY DO UDZIAŁU
W ŚRODKOWOEUROPEJSKIEJ KONFERENCJI

SUBSTANCJE CHEMICZNE
W ŚRODOWISKU PRZYRODNICZYM
w dniach 23-26 X 2013, w Hotelu Ziemowit w Jarnółtówku

Będzie to **dwudziesta druga z rzędu** konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotycząca różnych aspektów ochrony środowiska przyrodniczego. Doroczne konferencje ECOpole mają charakter międzynarodowy i za takie są uznane przez Ministerstwo Nauki i Szkolnictwa Wyższego. Obrady konferencji ECOpole ’13 będą zgrupowane w pięciu Sekcjach:

- **Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring**
- **SII Odnawialne źródła energii i jej oszczędne pozyskiwanie oraz użytkowanie**
- **SIII Forum Młodych (FM) i Edukacja prośrodowiskowa**
- **SIV Wpływ zanieczyszczeń środowiska oraz żywności na zdrowie ludzi**

Materiały konferencyjne będą opublikowane w postaci:

- abstraktów (0,5 strony formatu A4) na CD-ROM-ie,
- rozszerzonych streszczeń o objętości 5–8 stron w półroczniku *Proceedings of ECOpole,*
- artykułów: w abstraktowanych czasopismach: *Ecological Chemistry and Engineering*/*Chemia i Inżynieria Ekologiczna* (*Ecol. Chem. Eng.*) ser. A i S oraz w półroczniku *Chemistry – Didactics – Ecology – Metrology* (*Chemia – Dydaktyka – Ekologia – Metrologia*).

Termin nadsyłania angielskiego i polskiego streszczenia o objętości 0,5–1,0 strony (wersja cyfrowa) planowanych wystąpień upływa w dniu 15 lipca 2013 r. Lista prac zakwalifikowanych przez Radę Naukową Konferencji do prezentacji będzie sukcesywnie publikowana od 31 lipca 2013 r. na stronie webowej konferencji:

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Aby praca (dotyczy to także streszczenia, które powinno mieć tytuł w języku polskim i angielskim, słowa kluczowe w obydwu językach) przedstawiona w czasie konferencji mogła być opublikowana, jej tekst winien być przygotowany zgodnie z wymaganiami stawianymi artykułom drukowanym w czasopismach Ecological Chemistry and Engineering ser. A oraz S, które są dostępne w wielu bibliotekach naukowych w Polsce i za granicą. Zalecenia te są również umieszczone na stronie webowej Towarzystwa Chemii i Inżynierii Ekologicznej:

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Po konferencji zostaną wydane 5–8-stronicowe rozszerzone streszczenia wystąpień w półroczniku *Proceedings of ECOpole*. Artykuły te winny być przesłane do **1 października 2013 r.** Wszystkie nadsyłane prace podlegają zwykłej procedurze recenzyjnej.

Koszt uczestnictwa w całej konferencji wynosi 1600 zł (w tym 23% VAT) i pokrywa opłatę za udział, koszt noclegów i wyżywienia oraz materiałów konferencyjnych. Jest możliwość udziału tylko w jednym wybranym przez siebie dniu, wówczas opłata wyniesie 1100 zł i będzie upoważniała do uzyskania wszystkich materiałów konferencyjnych, jednego noclegu i trzech posiłków (śniadanie, obiad, kolacja).

Opłata dla magistrantów i doktorantów oraz młodych doktorów biorących aktywny udział w Forum Młodych może być zmniejszona do 1000 zł (w tym 23% VAT) przy zachowaniu takich samych świadczeń. Osoby te winny dodatkowo dostarczyć: rozszerzone streszczenia (5–8 stron) swoich wystąpień (**do 15.08.2013 r**.). Jest także wymagana opinia opiekuna naukowego.

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Wszystkie wpłaty powinny być dokonane na konto Towarzystwa Chemii i Inżynierii Ekologicznej w Banku Śląskim:

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i mieć dopisek ECOpole '13 oraz nazwisko uczestnika konferencji

Streszczenia oraz program konferencji zostaną wydane na CD-ROM-ie, który otrzyma każdy z uczestników podczas rejestracji. Program będzie także zamieszczony na stronie webowej konferencji

Po konferencji będzie możliwość opublikowania elektronicznej wersji prezentowanego wystąpienia (wykładu, a także posteru) na tej stronie.

Prof. dr hab. inż. Maria Wacławek
Przewodnicząca Komitetu Organizacyjnego
Konferencji ECOpole '13

Wszelkie uwagi i zapytania można kierować na adres:
Maria.Waclawek@o2.pl lub mrajfur@o2.pl
tel. 77 401 60 42
fax 77 401 60 51

GUIDE FOR AUTHORS

A digital version of the manuscript should be sent to:

Prof dr hab. Witold Wacławek,
Editor-in-Chief of Ecological Chemistry and Engineering A
Uniwersytet Opolski
ul. kard. B. Kominka 6
45–032 Opole
Poland
phone +48 77 401 60 42, +48 77 455 91 49
fax +48 77 401 60 51
email: maria.waclawek@o2.pl
mrajfur@o2.pl

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[1] Lowe DF, Oubre CL, Ward CH. Surfactants and cosolvents for NAPL remediation. A technology practices manual. Boca Raton: Lewis Publishers; 1999.

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